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# ABSTRACT

This investigation was undertaken to determine whether the use of paraformaldehyde powder for the sterile storage of gutta-percha cones is necessary, effective and safe. Gutta-percha cones from unopened manufacturer's packages were found to be sterile and to possess no inherent antimicrobial properties. Paraformaldehyde powder placed within the storage container was ineffective in sterilization of cones contaminated by bacterial endospores. Such storage did prevent contamination of cones by air-borne agents, but storage in a covered glass container without paraformaldehyde was equally effective. Formaldehyde was found to be adsorbed onto the surface of cones exposed to formaldehyde vapors. No significant increase in the level of formaldehyde in the operatory air was detected as a result of this storage method. It is recommended that this method of "sterile storage" be discontinued and efforts be directed at the prevention of contamination of cones during transfer from storage and preparation for obturation.

A MICROBIOLOGIC AND SPECTROPHOTOMETRIC INVESTIGATION

OF THE USE OF PARAFORMALDEHYDE POWDER IN THE

STERILIZATION OF GUTTA-PERCHA CONES

by

James R. Higgins, DDS

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Submitted to the Faculty of the Graduate School in partial fulfillment
of the requirements for the degree of Master of Science in Dentistry,
Indiana University School of Dentistry, 1985.

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INTRODUCTION

An essential element of most endodontic techniques is the removal of contaminants already within the root canal, and the prevention of contamination from outside the canal system. In the pursuit of an aseptic technique, dentists have adopted a variety of procedures, including sterilization of instruments, disinfection of contaminated surfaces of the operatory, use of the rubber dam, and thorough chemomechanical preparation of the root canal. Considerable care is taken in this effort, but the chain of asepsis can easily be broken if the gutta-percha used in obturation is not sterile.

Gutta-percha cones are often used without regard to whether they are sterile, and without ever having been sterilized after removal from the package. Few studies have reported on whether this is important. Moorer and Genet,[1] in a pilot study, tested whether gutta-percha cones are sterile as they are taken from the manufacturer's package. They aseptically opened gutta-percha containers and, in 8 of 9 trials, found their contents sterile. Fewer than 50 viable organisms were recovered from the contaminated container. Kos et al.[2] and Doolittle et al.[3] also tested gutta-percha fresh from the package, and found it sterile. Linke and Chohayeb[4] obtained similar results, although once the cones were exposed to the environment of the dental operatory, they did not remain sterile. Montgomery[5] found that cones taken directly from unopened containers were not sterile, but only 4 of 46 were contaminated. There are several possible sources of contamination once the packages are opened, including contamination during storage and preparation for use. No studies have been found which address the degree of contamination possible during storage. Doolittle and associates[3] demonstrated that even when a gutta-percha cone is sterilized at

chairside, it can still become contaminated in the few moments before it is used, unless proper precautions are taken. They emphasized the importance of the chain of asepsis.

Gutta-percha is not amenable to standard high temperature sterilization, since the cones are easily damaged by heat. In addition, the use of liquid sterilizing agents for storage of gutta-percha cones is cumbersome, due to possible spillage of the contents and the fact that the fluid must be changed frequently to maintain effectiveness. It has been claimed that prolonged storage in a liquid disinfectant is liable to soften the gutta-percha cones.[6]

This study investigated one possible method of sterile storage, the use of paraformaldehyde powder, to determine whether it is necessary and effective. It also sought to determine whether the patient or the dental office staff are exposed to significant levels of formaldehyde as a result of this storage method.

REVIEW OF THE LITERATURE

The importance of sterilizing gutta-percha cones prior to use has been recognized by numerous authors,[7-15] and many methods have been proposed. The major difficulty in the sterilization of gutta-percha is that the gutta-percha will not tolerate the heat of the autoclaving process.

This review will begin with the physical properties of gutta-percha, particularly its thermoplasticity, followed by the various methods that have been proposed for sterilizing gutta-percha, including the use of paraformaldehyde powder. The review will conclude with a summary of the development and use of formaldehyde in sterilization, the sources and hazards of formaldehyde exposure and government regulations thereon, and its use in dentistry.

## GUTTA-PERCHA

Gutta-percha is a product made from the latex of trees of the genus _Payena_ found mainly in the Malay Peninsula, Indonesia, and Brazil.[16] Its use as a molding material in those areas dates back to "a remote and undetermined epoch long before Western Civilization had any knowledge of its existence."[17] It was first introduced into England before 1656 when it was described as "mazer wood."[17] It was reintroduced into Europe in the 1840's under the name "gutta-percha."[17] Its ready pliability on heating, and its great elasticity led to its use in many diverse ways. These included the manufacture of corks, cements, thread, surgical instruments, shoes, pipes, boats, golf balls, and insulation for underwater telegraph cables.[7,17] The development of vulcanized rubber in the late 19th century led to the abandonment of gutta-percha in all but a few of its former uses.

The development of the first gutta-percha formulation for use in dentistry is credited to Dr. Asa Hill.[16] He patented the preparation, which consisted principally of bleached gutta-percha and carbonate of lime and quartz, in 1848. Gutta-percha was first used as a root canal filling material in 1867 by Dr. G. A. Bowman.[18] There are scant references to the development of gutta-percha as an endodontic material. In 1883 Perry claimed to have used a pointed gold wire wrapped with soft gutta-percha to fill root canals.[19] In 1887 S. S. White began to manufacture gutta-percha points.[20]

Development of the formulations used by various manufacturers has been shrouded in secrecy, and exact formulas are difficult to obtain. However, in 1977 Friedman et al.[21] reported their studies of the composition and physical properties of five brands of gutta-percha used in endodontics. Their analysis showed that the five brands contained nearly identical proportions of organic and inorganic components. There were differences in the composition of the two fractions, however. All contained gutta-percha (18.9% to 21.8%), zinc oxide (59.1% to 75.3%), metal (barium or strontium) sulfates (1.5% to 17.3%), and wax and/or resin (1.0% to 4.1%). The authors stated that gutta-percha is a thermoplastic substance, tending to be brittle at lower temperatures and ductile at higher temperatures.

Goodman et al.[17] have provided a thorough review of the molecular chemistry of gutta-percha. They pointed out that while rubber is a "cis" polymer of isoprene, gutta-percha is a "trans" polyisoprene and that it occurs in two forms. The "alpha" form of gutta-percha is that found in nature, while the "beta" form is used in most commercial preparations. The structures of isoprene, rubber, and gutta-percha are depicted in the following illustrations:

```
                                             CH3     H
                                              \     /
                        CH2    CH2     C=C    CH2    CH2
         CH3           /   \  /   \   /   \  /   \  /   \  /
          |          /     C=C     CH2     CH2     C=C     CH2
       H2C=C-C=CH2         /  \                    /  \
          |             CH3    H                CH3    H
          H

       isoprene                          natural
       monomer                           rubber

    -CH2    H   CH3   CH2-CH2    H      -CH2    H         beta
      \    /     \    /     \    /        \    /         gutta-
       C=C        C=C        C=C           C=C           percha
      /    \     /    \     /    \        /    \
    CH3    CH2-CH2    H   CH3    CH2-    CH3    CH2-CH2    H
                                                      \   /
        alpha                                          C=C
        gutta-percha                                  /   \
                                                   CH3     CH2-
```
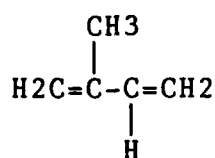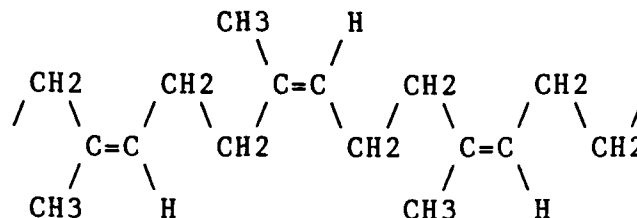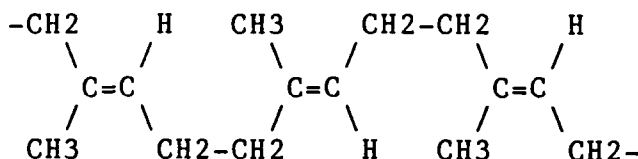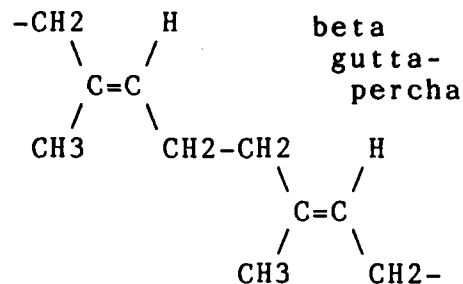
If the natural "alpha" form is heated above 65°C, it melts and becomes amorphous. If it is allowed to cool slowly (0.5°C/hour), it reforms in the "alpha" crystalline structure. If it cools normally, the "beta" gutta-percha forms. The "beta" form melts at 56°C. There is no apparent difference in the mechanical properties of the "alpha" and "beta" forms. Schilder et al.[22] determined the phase transition temperatures of dental gutta-percha compounds to be 42°C to 49°C for "beta" to "alpha" transition, and 53°C to 59°C for "alpha" to amorphous transition, depending on the specific compound tested. The temperatures used in various sterilizing methods (160°C for dry heat, 127°C for chemical vapor, and 121°C for saturated steam)[23] are all above the melting point of gutta-percha. Utilization of these methods in the sterilization of gutta-percha cones would render the cones useless. This fact has led many investigators to seek alternative means of sterilizing gutta-percha.

# STERILIZATION OF GUTTA-PERCHA

The alternatives proposed for the most part fall into two general categories: cold sterilization by a liquid agent, or gaseous sterilization.

Many liquid agents have been advocated for the sterilization of gutta-percha. Zephiran and Zephiran Chloride have been recommended by many authors.[6,7,12,13] Doolittle et al.[3] found that Zephiran, tincture of Metaphen (50%), and ethyl alcohol (70%) were effective disinfectants for gutta-percha when placed in the solutions 15 minutes. They did not test the effectiveness of these agents against spores, however. Linke and Chohayeb[4] found that a five-minute exposure to Zephiran (0.53%), hydrogen peroxide (3%), or sodium hypochlorite (as low as 1.125%) sterilized gutta-percha contaminated by a variety of microorganisms including fungi, yeasts, vegetative forms of gram (+) and gram (-) bacteria, and Bacillus subtilis. They did not state whether spores were tested. After a five- minute soak, povidone-iodine (Betadine), eugenol, chloroform and iodine were not effective against the contaminants. Montgomery,[5] however, found that Betadine was an effective decontaminant after six minutes of exposure, though not at four minutes. He did not test against spore-formers.

Suchde et al.[24] reported that Savlon (a combination of chlorhexidine gluconate and cetavlon cetrimide) was effective against B. subtilis spores and Candida krusei, as well as staphylococci and streptococci. Even spores were killed after immersion for 30 seconds to one minute. Several authors have recommended tincture of Metaphen.[15,25,26] Other recommended agents include thiomersal,[9] glutaraldehyde,[27] alcohol,[11] and sodium hypochlorite.[28]

Frank and Pelleu[27] found that sodium hypochlorite (5.25%), and Sporicidin (aqueous 2% glutaraldehyde, 7% phenol, and 7% wetting agent) were effective in sterilizing gutta-percha contaminated with B. subtilis spores after exposures of one minute and five minutes, respectively. Cidex (aqueous 2% glutaraldehyde) reduced the contamination level by 99.9% after 15 minutes. They stated that sodium hypochlorite appears to be the solution of choice for sterilizing gutta-percha cones, with Sporicidin an acceptable alternative. Senia et al.[28] also found that 5.25% sodium hypochlorite killed B. subtilis spores after a one-minute immersion.

In 1978 the ADA Council on Dental Therapeutics reported that quaternary ammonium compounds are not acceptable for disinfection of instruments and environmental surfaces in dentistry.[29] The Council stated that these agents were not effective in killing Mycobacterium tuberculosis and enteroviruses (nonlipid viruses) within 30 minutes. The compounds were not effective against Clostridium species, other spore-forming bacteria, poliovirus 1, and type B hepatitis virus. Quaternary ammonium compounds previously accepted were benzalkonium chloride (Zephiran), dibenzalkonium chloride, and cetyldimethylethyl ammonium bromide (Cetylcide). Also found ineffective were 70% ethanol, 90% isopropanol, and the phenols. The Council approved aqueous solutions of formaldehyde (3% and 8%), chloride compounds (including commercial bleach), and glutaraldehyde (aqueous 2%).

Gaseous sterilization of gutta-percha has also been recommended. Senia et al.[30] found that the vapors from formocresol killed a variety of microorganisms, including B. subtilis, with which they contaminated gutta-percha cones. The minimum exposure time tested was 16 hours, however. Ethylene oxide gas has been recommended, but the long cycle time required at appropriately low temperatures makes it impractical for use in

the dental office.[23,31] Ehrmann and associates[32] suggested the use of propylene oxide gas. They found that it killed Bacillus stearothermophilus spores in as little as 3.5 hours. They stated that in practice the cycle is usually six hours.

Paraformaldehyde powder has been advocated for the sterilization and sterile storage of dental instruments and materials. In 1956 Castagnola and Orlay[33] gave passing notice to the use of "formaldehyde powder or tablets" placed in a container for the storage of sterilized instruments. Kantorowicz[34] described a system of sterilization and storage of paper points and gutta-percha cones using paraformaldehyde tablets in a screw-top jar. Staphylococcus pyogenes, Streptococcus viridans, B. subtilis, Neisseria catarrhalis, and Candida albicans inoculated onto paper points and gutta-percha cones all showed no growth when incubated after four hours exposure to the formaldehyde gas in the jar. The same materials placed in a jar containing one paraformaldehyde tablet were found to be sterile when tested after 120 days of continuous use. Buchbinder[35] briefly described his in-office use of paraformaldehyde powder for sterilization and ready storage of cotton points and gutta-percha cones. He placed paraformaldehyde powder in one section of a 4" petri dish and placed contaminated gutta-percha cones and cotton points in the other sections of the same dish. B. subtilis was killed in four hours with dry paraformaldehyde powder or in three hours with moistened powder. Staphylococcus albus was killed in two hours with dry powder and in "less time" with moistened powder.

## FORMALDEHYDE

Formaldehyde, HCHO, is a colorless gas that condenses to form a liquid of high vapor pressure that boils at $-19^{\circ}$C. It forms a crystalline solid at $-118^{\circ}$C. In the monomeric form, its molecular structure is as shown below.[36-38] It has a pungent odor and is highly irritating to the exposed membranes of the eyes, nose, and upper respiratory tract.[39] It is also intensely irritating to the skin, and is a skin sensitizer.[40,41]

$$\begin{array}{c} H \\ \diagdown \\ C = O \\ \diagup \\ H \end{array}$$
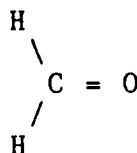
In the pure liquid form at temperatures below $-90^{\circ}$C, it polymerizes slowly. Above this temperature, or in the presence of moisture, polymerization occurs readily, and formaldehyde can thus be kept in a pure monomeric state for only a limited time.[36,42,43]

Formaldehyde gas is easily soluble in water. Formaldehyde in aqueous solution is present principally as methylene glycol, a monohydrate, and as a series of low molecular weight polymeric hydrates (polyoxymethylene glycols) having the general formula

$$HO\text{-}(CH_2O)_n\text{-}H.\text{[36,44,45]}$$
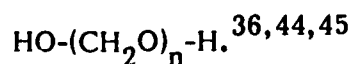
Aqueous formaldehyde is available commercially as formalin, which contains 37 to 50 per cent formaldehyde by weight.[36,39,40] This is a clear

solution which is slightly acidic and has the strong, pungent odor of formaldehyde. Formalin contains a small amount of formic acid and a considerable amount of methanol. The methanol hinders polymerization by breaking down the high molecular weight polymethylene glycols, forming hemiacetals, which are more soluble than the glycols.[36] A small amount of monomeric formaldehyde is present, but its concentration is very low.[36] The formation of formaldehyde polymers is as follows:[44]

$$CH_2O + H_2O = HOCH_2OH \text{ (methylene glycol)}$$

$$HOCH_2OH + (n-1)HCHO = HO(CH_2O)_nH \text{ (polymer)}$$
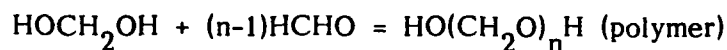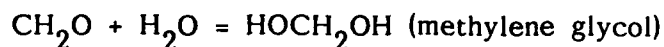
Low formaldehyde concentrations favor methylene glycol, and high concentrations favor the polyoxymethylene glycol polymers.[36]

## PARAFORMALDEHYDE

Paraformaldehyde is a colorless solid. It is available in a granular form or as a white powder with an odor characteristic of formaldehyde. It is a linear polymer of formaldehyde, with the general formula $HO-(CH_2O)_n-H$ with n=8 to n=100.[36,46] The linear structure is represented below:

```
      H    H    H          H    H    H
      |    |    |          |    |    |
 HO - C -O- C -O- C -O-  // -C -O- C -O- C - OH
      |    |    |          |    |    |
      H    H    H          H    H    H
```
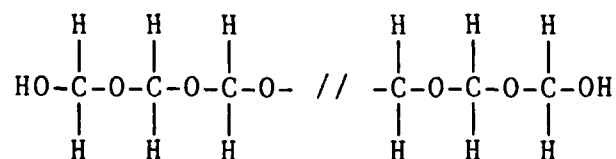
Paraformaldehyde melts over a wide temperature range, 120-170°C, which is directly related to the degree of polymerization.[46] As n increases, the melting point increases above 120°C. At room temperatures it gradually vaporizes largely as a monomeric formaldehyde with some water formation.[39,47] The rate is increased by increasing the temperature or relative humidity.[39,48]

Since the formaldehyde released from paraformaldehyde is nearly pure monomeric form, it is preferred as a source of formaldehyde over formalin, which contains several impurities to prevent polymerization. It is also the preferred source of formaldehyde gas for sterilization and disinfection.

## HISTORY OF FORMALDEHYDE AND PARAFORMALDEHYDE

Research conducted on acetaldehyde in 1835 by Liebig became the foundation for understanding the chemical nature of aldehydes.[49] Over the next two decades other aldehydes were discovered and recognized as belonging to a group of chemical compounds with the common general formula $C_nH_{2n+1}CHO$. The name aldehyde is derived from the term "alcohol dehydrogenatus" which signifies that aldehydes are derived from alcohols by removal of hydrogen.[49] They are usually named from the carboxylic acids with the same number of carbon atoms, such as acetaldehyde, $CH_3CHO$, butyraldehyde, $C_3H_7CHO$, et cetera.[49] Propionaldehyde, butyraldehyde, and isovaleraldehyde, among others, had been discovered well before 1859, when formaldehyde (n=0 in the above formula) was first prepared by A. M. Butlerov.[47,50] He described the various forms of formaldehyde and its reactions.[50] The preparation was carried out by hydrolyzing methylene

acetate. In 1868 A. W. Hoffman prepared formaldehyde by passing a mixture of methanol vapors and air over a heated platinum spiral.[47] This is the basis for the modern methods of formaldehyde production.[50] Hoffman identified formaldehyde as the first member of the aldehyde series.[50]

Butlerov also was the first to prepare paraformaldehyde. In 1859 he obtained it by vacuum distillation of formaldehyde solutions, but he erroneously concluded that it was dioxymethylene $(CH_2O)_2$.[46] Hoffman showed that Butlerov was in error, but incorrectly labeled it a trimer, and called it trioxymethylene. He found that upon vaporization it yielded monomeric formaldehyde.[46] The name paraformaldehyde was first used by Tollens and Mayer[46,51] in 1888. They applied it to the polymeric residue after formaldehyde solutions are evaporated. In 1890 Losekann[46] discovered that this polymer contained combined water, and reported that it was a polymeric hydrate with the formula $(CH_2O)_6$-$H_2O$. In 1897 Delepine[46] determined an average formula for formaldehyde, which he called a mixture of polymeric hydrates, to be $(CH_2O)_8$-$H_2O$. He stated that it was formed by the condensation of methylene glycols as indicated by the equation

$$nCH_2(OH)_2 = (CH_2O)_n\text{-}H_2O + (n\text{-}1)H_2O.$$
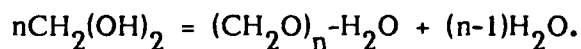
In its essentials, this statement is consistent with the conclusions of modern chemists who give the formula as

$$HO\text{-}(CH_2O)_n\text{-}H.^{46,51}$$
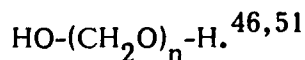
Paraformaldehyde is produced today, in general, by distillation and concentration of formaldehyde in solution. This continues until solidification or precipitation of polymers occurs. The resulting product contains 91% to 99% formaldehyde, the remainder being combined water.[51]

## FORMALDEHYDE STERILIZATION

In a review article on the early development and testing of formaldehyde sterilization, Nordgren[47] pointed out that the bactericidal properties of formaldehyde were described by Loew in 1888. In 1892 Trillat dealt with its usefulness in disinfection. In further investigations in 1892 and 1893, other authors verified that formaldehyde has a strong bactericidal effect in aqueous solution as well as gaseous form. Interest in the use of formaldehyde for sterilization increased, with numerous investigations being undertaken between 1895 and 1910.

As reviewed by Nordgren, room disinfection with formaldehyde gas was first described in 1894 and 1895 by Philip et al. and Bardet et al. They concluded that the results were satisfactory, on the whole. Oehmichen discovered in 1894 that the capacity of formaldehyde to penetrate porous material is rather limited. Ordinary writing paper appeared to completely protect spores from the gas. In the following year Pottevin found that better results were achieved by increasing the temperature or by moistening the objects to be sterilized. In 1896 Roux and Trillat, on the other hand, reported that dry gas produced better results than were obtained with moist conditions. Struver in 1896 experimented on levels of formaldehyde saturation of the air in rooms being disinfected. He found that an original concentration of 1mg/liter (about 800 parts per million[ppm]) failed to kill anthrax spores on silk threads; concentrations of 1.5 mg/liter killed typhus bacteria protected by six layers of flannel, but anthrax spores were still viable. If the original concentration was 2 mg/liter, freely exposed anthrax

spores were killed, but even at 2.5 mg/liter (about 2,000 ppm), spores that were protected by several layers of flannel remained viable.

By 1897, paraformaldehyde was used as the source for formaldehyde gas for disinfection. Nordgren noted that the method was tested by many authors of the period. Gehrke in 1898 found that formaldehyde so generated could penetrate cotton wool. Harrington, however, concluded in 1897 that it had very limited penetration capacity, and that it should not be considered to have more than a fairly superficial disinfectant effect.

According to Nordgren, the earliest attempts to sterilize surgical instruments by formaldehyde gas were conducted between 1895 and 1900. In 1896 Janet developed a method in which the instruments were placed upon metal wiring in a closed box with paraformaldehyde powder sifted onto the bottom of the box. Sterilization was done at room temperature, humidity, and pressure. This method, though widely used, was controversial due to the inconsistent results obtained. Auerbach and Pluddemann, and Auerbach and Barschall in a series of experiments from 1904 to 1909 showed that an equilibrium exists between formaldehyde and paraformaldehyde, which they described with the following equation:

$$nCH_2O = (CH_2O)_n.$$
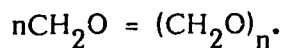
They found that an increase in the concentration of formaldehyde or a decrease in temperature shifted the equilibrium to the right. In 1914, they stated that below $100^{o}C$ the gas consisted almost exclusively of polymerized molecules. They assumed that water vapor has a dissociative effect on the polymerized gas molecules, thus accounting for increased effectiveness with increased humidity. They felt it was never appropriate to assume the whole quantity of gas produced is effective.

At about this time, other more effective and less controversial methods of sterilization became available,[52] and with them came a decrease in interest in formaldehyde. However, as Nordgren stated in his review, investigations continued. Blair and Ledbury found in 1925 that the vapor pressure curve of the depolymerization of paraformaldehyde corresponds to that of a 35% aqueous solution of formaldehyde. In 1934 Huss and Maunsbach investigated methods of sterilizing dental instruments, using various formaldehyde sterilizers. They found no evidence of sterilization in any of the systems tested. In a comprehensive study of the factors affecting the efficacy of formaldehyde gas sterilization, Nordgren[47] attempted to clarify the conflicting results of earlier experiments. He studied ways in which temperature, concentration, humidity, and the physical and chemical protection of the organisms affected the sterilization process. The experiments were conducted in a controlled environment: the atmosphere contained measured concentrations of formaldehyde and water vapor at closely regulated temperatures. The test objects were lengths of glass tube onto which a suspension of the test organisms was dried. After exposure to the formaldehyde, the tubes were washed with sodium sulphite to remove residual formaldehyde. The remaining bacteria were dispersed in normal saline and plated on agar for colony counts. Nordgren concluded that gaseous formaldehyde obtained through the vaporization of paraformaldehyde at room temperature is at least 90% monomeric, with up to 10% polymers. He found that the rate of killing was dependent on the combination of temperature, humidity, and formaldehyde concentration. The killing rate increased as the temperature, humidity, or vapor pressure of formaldehyde increased. There was, however, little increase in the rate as the relative humidity increased from 50% to 90%.

Nordgren confirmed the findings of Blair and Ledbury, noted above. Gross wetting of the objects to be sterilized was found to retard killing, as did protection of spores by coating them with blood or sputum. All bacterial vegetative forms were killed within one hour at room temperature with 90% to 100% relative humidity if the air was one-half to three-fourths saturated with formaldehyde. These conditions could not be achieved by the Janet method, and Nordgren therefore introduced his own improvements of that method. These consisted of directing a constant air current over the paraformaldehyde, and controlling the humidity. He concluded that paraformaldehyde evaporates so slowly without the aid of a steady air current passing over it, that it is of little practical use, and this slow evaporation may account for the poor reputation of "dry" formaldehyde as a disinfectant. He also stated that all sterilizers of the Janet type which are constructed for general purposes should therefore be condemned.

Nordgren's paper did not halt the use of formaldehyde in sterilization. It continued to be popular in the disinfection of bedding in hospitals, of articles made and sold by patients with tuberculosis,[53] and of laboratory rooms, as well as in some veterinary applications.[45] Other uses were in the fumigation of wool, and in the sterilization of certain types of surgical and medical equipment in small cabinets.[45] However, the tendency was more toward disinfection and less toward sterilization.

Because of this continued use, formaldehyde was studied by the Committee on Formaldehyde Disinfection of the Public Health Laboratory Service of Britain between 1950 and 1958.[53] They used lengths of white cotton thread which were contaminated, in early tests, with a coagulase-negative micrococcus (NCTC 7944). In later tests the cotton was contaminated with M. tuberculosis, B. subtilis, or smallpox virus.

Suspensions of the contaminant were dried onto the cotton. In some cases the contaminants were protected by a coating of 1% gelatin, or 90% horse serum. The disinfection tests were carried out in widemouth jars with atmospheres of known relative humidity and known concentrations of formaldehyde gas. The threads were attached to the lid and suspended in the formaldehlyde vapor. The experiments were carried out at several temperatures from $0^{o}$C to $40^{o}$C. At intervals, threads were removed from the jar and cultured. A duplicate set of threads that were treated identically but without exposure to formaldehyde served as controls. The results showed a direct relation between formaldehyde concentration and the killing rate. This confirmed Nordgren's finding that little increase in the rate of killing resulted from increasing the relative humidity above 50%, though there was an optimum rate at 80% to 90%. Horse serum inhibited killing more than gelatin. The authors found these results very difficult to reproduce, and stated that this was one of the "most disconcerting features of disinfection by formaldehyde." It was impossible to predict the efficacy with any real confidence under any given set of conditions. They therefore concluded that disinfection by formaldehyde vapor should be used only when no other method is available.

Today, the use of formaldehyde in disinfection and sterilization generally conforms to this stricture, and is usually limited to certain instruments, materials, and objects which will not stand up to the conditions imposed by other sterilization procedures.

In a brief review, Phillips[45] stated in 1968 that during the course of sterilization by formaldehyde gas, it tends to condense onto the surface of the objects being sterilized and the walls of the container. This decreases the formaldehyde gas concentration in the container. In order to maintain a

high enough concentration, an excess of paraformaldehyde must be maintained, and the temperature must be at least 20°C, preferably higher. Phillips further stated that if the proper conditions can be maintained, "within an hour or two vegetative bacteria will be destroyed, but the space must be kept closed for as long as 12 hours if a high concentration of bacterial spores is present." This, he found, worked well for exposed surfaces, but did not sterilize surfaces covered in any manner. It was not necessary to hermetically seal the container. One major disadvantage he mentioned was the fact that prolonged airing, often several days, is required to remove the adsorbed surface film of polymer, which continues to release formaldehyde gas slowly.

In 1969 Taylor et al.[51] studied the use of paraformaldehyde gas to sterilize sensitive electronic equipment which had been contaminated by Clostridium botulinum. They heated measured amounts of paraformaldehyde powder to 232°C, to liberate the gas. Two laboratory rooms and a mobile laboratory trailer were seeded with B. subtilis spores and Serratia marcescens organisms. Contact time in the tests was one to two hours after dissemination of the gas. The room temperature was maintained at 31°C with 55% relative humidity. The contaminated sites included glass, rubber, plastic, stainless steel, painted surfaces, copper, aluminum, vinyl sheeting, and various types of electronic laboratory equipment. In all tests, the microorganisms were killed. Spores were killed on surfaces contaminated with $10^7$ spores/ml by exposure to the formaldehyde gas produced by a paraformaldehyde concentration of 0.3 gram paraformaldehyde powder per cubic foot of volume for a one-hour contact period. In similar tests, C. botulinum was killed both in liquid suspension and as dry material on flat surfaces. During the tests, no visible residue of formaldehyde polymer was

found on the sterilized materials, except when the relative humidity was deliberately increased to 100%.

In 1975 El-Gammal and Mostafa[54] reported successful sterilization of ophthalmic surgical instruments, cotton, and lint which had been contaminated with Staphylococcus aureus, Streptococcus faecalis, Salmonella typhi, or other bacteria. They did not test against spore-formers. The contaminated materials were placed into an air-tight glass container 17 cm in diameter, containing one paraformaldehyde tablet of unstated weight. It was found that the instruments, cotton, and lint were sterilized after at most five hours of exposure. They recommended at least six hours of exposure before using previously contaminated materials.

In 1977 Kurze et al.[55] investigated the use of formaldehyde gas in the sterilization of surgical microscopes. The instrument was cleaned with Betadine, and two surfaces were then contaminated, one with S. aureus, and the other with Pseudomonas aeruginosa; they did not test against spores. The instrument was sealed in a polyethylene bag containing 10 grams of paraformaldehyde powder for 48 hours at room temperature. When the bag was opened, only a weak odor of formalin was noted. Smears from the designated surfaces were plated, resulting in abundant growth of both test organisms. They repeated the experiment eight times, and in no case did sterilization occur. Even when formaldehyde gas produced by the heating of paraformaldehyde powder was sealed in the bag, sterilization did not occur. They concluded that this method of sterilization was ineffective.

Winn et al.[56] reported on the use of "paraformaldehyde gas" to decontaminate a laboratory room. In 1982 they compared the results obtained in decontamination of a petri dish that contained a suspension of Legionella pneumophilia in phosphate-buffered saline, a sterile glass slide on

which a saline suspension of L. pneumophilia had been dried, and a B. subtilis spore strip. In another experiment, S. marcescens and Escherichia coli were also tested. The room was sealed, and paraformaldehyde gas was generated from fine powder on a hot plate. The relative humidity was maintained at 70% to 80% at a constant temperature of 21°C. The amount of powder used was either 0.3 g per cubic foot or 0.6 g per cubic foot. The B. subtilis strips were sterilized, and though the saline suspensions of the L. pneumophilia, E. coli, and S. marcescens were not sterilized, they were killed efficiently after exposure to the gas. There was a significant reduction in bacteria present, from $10^9$ to less than 10 colony-forming units (cfu) in as little as five hours with 0.3 g powder per cubic foot. No killing was demonstrated in the dried suspensions. The authors speculated that the vegetative bacteria might have been protected from the formaldehyde gas by inclusion in large inorganic crystals that form as the salt suspension dries.

The most recent investigation on the use of paraformaldehyde in sterilization was a series of articles reported by Wigert et al.[57-59] from 1977 to 1982. They studied the antimicrobial effects of paraformaldehyde, the composition of the gaseous formaldehyde released by paraformaldehyde tablets, and the use of paraformaldehyde tablets in medical institutions. S. aureus, gram(-) spore-forming rods, yeasts, and fungi, as well as some Bacillus species, were placed on various surfaces such as metal, blotting paper, and cotton cloth, exposed to the formaldehyde vapors, and the killing time was noted. They found that formaldehyde gas is germicidal, and that the effect is faster on smooth surfaces than on materials that are rough or inaccessible. Vegetative bacteria, fungi, and yeasts were killed very easily, while spore-formers were significantly more resistant. The greatest

germicidal activity was noted at 80% relative humidity, with a hermetically sealed system.

In contrast to the findings of the Committee on Formaldehyde Disinfection, Wigert et al. found that a significant decrease in killing time occurred as the relative humidity was increased from 55% to 80%. They reported that the maximum concentration of formaldehyde liberated by paraformaldehyde at $21^{o}C$ was 2.0 mg/liter (about 1600 ppm). The speed at which the saturation point was reached was found to be very dependent upon the amount of paraformaldehyde used per unit of volume. They found that an optimum result was obtained using three one-gram tablets for a 130 cc container. For example, the maximum concentration was reached in eight hours using one tablet, six hours using two tablets, and one hour using three tablets. The killing rates were fastest if three tablets were used, and slowest if only one was used. The gas released within the container was identified by mass spectrography as monomeric formaldehyde.

In a test of the efficacy of the method on contaminated instruments, Wigert et al. found that results were dependent on too many variables to be reliable. The more frequently the container was opened, the more likely it was that contamination would occur. In one investigation, 20% of the sterilized instruments placed in a cabinet containing paraformaldehyde tablets became contaminated within eight hours of use in one dental office. The contaminants were for the most part spore-formers. The authors did recommend its use for limited purposes, however. Since even Bacillus spores were killed after 15 hours of constant exposure, they recommended that paraformaldehyde tablets be used for targeted antimicrobial procedures in medical practice. The guidelines they recommended are: (1) for germ count reduction, use 1 g per 100 cc for at least three hours, (2) for disinfection of

weakly contaminated or not grossly soiled instruments, use 10 g per 100 cc for at least three hours, (3) for sterilization, use 10 g per 100 cc for at least 15 to 24 hours, and (4) for the storage of unpacked sterilized instruments, use 1 g per 100cc. For all uses as close to 80% relative humidity as possible should be maintained.

## EFFECT OF FORMALDEHYDE ON MICROORGANISMS

Though formaldehyde has been shown to be highly inconsistent in its effectiveness as a sterilizing agent, there is no doubt it has significant microbicidal properties, as discussed above. The basis for these properties is found in the propensity of formaldehyde to react with other chemical compounds. It is the most reactive of the one-carbon compounds, spontaneously polymerizing with itself, and undergoing a host of condensation, oxidation, reduction, and other reactions.[60] It reacts readily with a wide variety of compounds containing an available hydrogen atom, including organic nitrogen compounds such as amines, amides, amino acids, nucleic acids, and proteins,[61] as well as compounds containing hydroxyl, carbonyl, SH groups, and aromatic rings.[36,43] Formaldehyde reacts with these compounds in a two step process.[36,43,62,63] In the first step, formaldehyde binds to the reactive hydrogen of the compound to form a hydroxymethyl (methylol) compound:

$$-NH_2 + CH_2O = -NH-CH_2(OH).$$
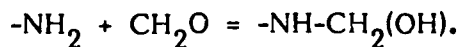
This product has been shown to be unstable, and easily reversed by hydrolysis.[43,63] The methylol compound then reacts with an additional

reactive hydrogen atom to form a methylene bridge:

$$-NH-CH_2(OH) + RH = NH-CH_2-R + H_2O.[36,43,62]$$
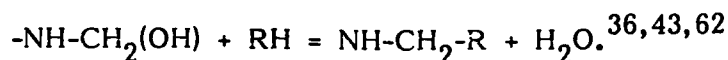
Chemical evidence of this reaction was provided by Nitschmann and Hadorn[64] who showed that a loss of water accompanied the addition of formaldehyde to casein. The methylene bridges are much more stable than the methylol compounds.[63] Fraenkel-Conrat and Olcott[65] found that this type reaction can occur at room temperature and at physiologic pH, between formaldehyde and simple primary and secondary amines and amides, within 24 to 48 hours. They stated that the primary reaction was the formation of methylol amines. The methylene condensation permits the introduction of simple amines and amides into proteins, and may also result in the stable introduction of reactive cyclic compounds.[66]

Formaldehyde has been shown to disrupt cell division. In 1954 Fraenkel-Conrat[67] concluded that formaldehyde inactivation of viruses is due to the action on the nucleic component rather than on the protein. In a series of reports, Neely[68-70] demonstrated that the biological activity of formaldehyde on Aerobacter aerogenes, and Pseudomonas aeruginosa was present only when the organisms were actively metabolizing and dividing. He showed that the initial action is to inhibit cell division, causing death through unbalanced growth. The other action is the formation of 1,3-thiazane-4-carboxylic acid from homocysteine. This compound prevents the synthesis of methionine which is an essential metabolite in cytoplasmic synthesis. Thus, he stated, the remainder of the action of formaldehyde is inhibition of both cytoplasmic and nuclear synthesis. The net result was considered bacteriostatic since once the formaldehyde was consumed the microorganisms resumed normal metabolism.

Chanet et al.[71] in 1975 confirmed the effect on cell division. The authors found that in random cultures of _Saccharomyces cerevisiae_, those in the stationary phase were more resistant to killing than those with exponentially growing cells. Formaldehyde induced intra- and intergenic recombinations, and maximum sensitivity occurred during the end of the $G_2$ phase of division.

Sentein[72] stated that formaldehyde acts as an antimitotic substance at lower concentrations, and as a fixative at higher concentrations. He noted arrested mitoses that were characterized by the absence of spindles and astral fibers, immobilizing the chromosomes in the equatorial region. The animal mitoses were more easily disrupted than vegetable. The author postulated that the mechanism of action of formaldehyde was via the "binding of two or several microtubule subunits by one molecule of the active substance...to form storage structures (which) would prevent the discharge of these microtubule subunits and consequently (prevent) the construction of microtubules."

In summary, it is not known whether any one action of formaldehyde is responsible for its microbicidal action. It is probably due to a combination of reactions between this highly reactive compound and the active hydrogen molecules on the various biochemical components of the microorganisms.

## FORMALDEHYDE EXPOSURE

Man is exposed to formaldehyde on a daily basis through a wide variety of sources. Formaldehyde has been called "primeval" and "ubiquitous" in its occurrence.[60] It has been found to exist in interstellar space.[73] It was

a prime component of the earth's early atmosphere[74,75] and is postulated to have been of major importance in the origin of life on earth.[76]

Formaldehyde plays a key role in the metabolic processes of virtually all living creatures. It is a normal metabolic product in most life forms and a vital precursor in the synthesis of other biochemicals essential to life.[61]

In small quantities it is rapidly metabolized.[77] The major route of metabolism appears to be oxidation to formic acid, followed by further oxidation to carbon dioxide and water.[78] The carbon dioxide is released in part via the lungs.[78,79] The enzyme formaldehyde dehydrogenase and various liver microsomal enzymes have been shown to catalyze the reaction in humans.[80] Though physiologic quantities of formaldehyde are readily metabolized, large exposures to formaldehyde can not be adequately processed, allowing the formaldehyde to react with various chemical components of the body, producing many adverse effects.

The sources of exposure to formaldehyde are both numerous and diverse. Occupational exposure to formaldehyde is common in many industries. In 1976 the National Institute for Occupational Safety and Health (NIOSH) identified over 60 occupations in which workers could potentially be exposed to formaldehyde.[81] It is estimated that between 5 and 10 billion pounds of formaldehyde per year are manufactured worldwide.[61] The manufacture of a wide variety of resins, such as urea-formaldehyde, accounts for the majority of uses of formaldehyde.[61,82] Formaldehyde is also used in agriculture as a seed, bulb, and root disinfectant, and in fertilizers; it is used in chemical analysis, in cosmetics, in deodorization, disinfection and fumigation, and in commercial dyes. Other applications are for embalming and preservation, processing of anatomical and pathologic specimens; in explosives, fireproofing, insecticides, leather, paper manufacture, and in the

refining of hydrocarbons. Formaldehyde is used as a reagent in the preparation and synthesis of medicinal products, and is used in the preparation of vaccines. It is also used in the production of silver mirrors; in electroplating and photography; in the production of rubber, solvents, plasticizers, and surface-active agents, as well as permanent-press clothing.[82]

Workers in plants which manufacture formaldehyde, or any of the formaldehyde resins are exposed to levels of formaldehyde as high as 10 ppm and occasionally as high as 30 ppm.[83] Studies of workers in funeral homes have indicated that exposure levels may reach as high as 5.26 ppm.[84] Formaldehyde may also affect those not involved in its manufacture or use. It has been found to be a major component of incinerator residues and Los Angeles smog.[85] It is also found in great abundance in cigarette smoke[86] and automobile exhaust.[85] In the home, individuals are exposed through many of the previously mentioned sources; however, some of the most significant sources of exposure are urea-formaldehyde insulation, and the release of formaldehyde from bonding agents in some plywoods and chipboards. Additional sources of exposure are through the resins used as molding compounds for the manufacture of dinnerware, appliances, and telephones.[87] Typical concentrations of formaldehyde from various sources are:[87,88]

| | | |
|---|---|---|
| Los Angeles smog | up to | 0.16ppm |
| Cigarette smoke | up to | 40.0ppm |
| Air in homes with urea-formaldehyde foam insulation | 0.4 to | 8.1ppm |
| Air in mobile homes with particle board components | 0.03 to | 2.5ppm |
| Fertilizer production | 0.2 to | 1.9ppm |
| Hospital autopsy room | 2.2 to | 7.9ppm |
| Plywood industry | 1.0 to | 2.5ppm |

## HEALTH EFFECTS OF FORMALDEHYDE EXPOSURE

The studies which resulted in the foregoing information were undertaken as a result of the ever-increasing concern for the possible ill effects of exposure to formaldehyde. There has been a gradual shift away from the original concern, which was mainly over the irritational and allergic effects of formaldehyde from contact with the skin or breathing the vapors. Though there is still reason to guard against these effects, today the emphasis is centered on the mutagenic and possible carcinogenic effects of exposure to formaldehyde.

The first reports of health hazards of formaldehyde came from studies of workers involved in the production or use of formaldehyde in its various forms. In 1945 the Industrial Hygiene Research Laboratory of the National Institutes of Health published a review of formaldehyde toxicity and potential dangers, which summarized some of the early research on the hazards of industrial exposure to formaldehyde.[89] According to the report, Meyer had found in 1893 that topical application of aqueous solutions of formaldehyde to the eyes caused severe irritation of the conjunctiva, lacrimation, keratitis, and superficial ulcers in animals. Several authors cited in the NIH review noted that in sensitive workers, exposure to formaldehyde or its vapors produced dermatitis of the face, neck, and arms as well as other parts of the body. These early writers also reported that the dermatoses were sometimes due to an acquired sensitivity to the formaldehyde, and were characterized by reddening and infiltration of the skin, which could show numerous vesicles and cracking. In the 1945 report, the effect of formaldehyde ingestion was also reviewed. Animal studies had shown that ingestion of formaldehyde caused a variety of toxic changes. These included:

(1) an initial stimulation and subsequent depression of the respiration, (2) a digitalis-like effect on the heart by small doses with larger doses causing a drop in blood pressure, and (3) severe inflammation of the stomach, duodenum, and jejunum. Human studies had demonstrated that ingestion of small amounts of formaldehyde with food has a delayed effect, and after several days eventually produces nausea and vomiting.

Several cases of fatal formaldehyde poisonings were described in the review. It was noted that with the ingestion of large doses, death may follow in 15 minutes to one or several hours, while with smaller quantities, the patient may suffer for several days. With oral administration, symptoms include severe pain from the gastrointestinal tract. The severity depends on the dose and the contents of the stomach at the time of ingestion. With diluted solutions, only superficial inflammation may result. Coagulation of tissue, dyspnea, loss of consciousness, circulatory collapse, and acute renal failure have resulted from the ingestion of concentrated solutions.[40] Postmortem findings may include fixation of the tissues of the stomach and intestines, inflammation of the esophagus, larynx, and lungs, as well as acute tubular necrosis of the kidneys.[40,90] In non-fatal cases the patients may undergo similar symptoms, but finally recover after suffering for some time from gastrointestinal disturbances or kidney injury.

The irritating effects of formaldehyde vapor were also reported in the 1945 review. Exposure to formaldehyde vapors was known to cause irritation of the mucous membranes of the eyes and respiratory tract. In animal experiments, prolonged exposure to low concentrations caused inflammation of the upper respiratory tract, emphysema, and congestion of the lungs. Recent studies have also noted that gastrointestinal effects,[91] kidney damage,[90] and dermatitis[92] may result from exposure to the vapors. No information was

available in 1945 with regard to the relation between the concentrations of formaldehyde in the air and the toxic symptoms in man. The report noted that Flury and Zernick in 1931 had demonstrated that concentrations of 20 ppm caused definite irritation of the mucous membranes of the eye and upper respiratory tract. Barnes and Speicher in 1942 had found that 10 ppm could cause distinct irritation, but 5 ppm was found to have no such effect. Weger in 1927 had stated that workers engaged in the manufacture of phenol-formaldehyde resins were subject to headache, weakness, sensory disturbances, irregular perspiration, and fluctuations of the body temperature. Baader in 1932 noted lacrimation, cough, chest congestion, rapid pulse, "hammering headache in the temples", and pressure in the kidney area in workers engaged in similar operations. In 1935 Ludwig had described a case of "bronchitis obliterans" in a patient following inhalation of dust from phenol-formaldehyde resins. More recent investigations of the irritant effects of formaldehyde have shown that the most common complaints are eye, nose, and throat irritation, and sensitivity to the odor. The odor was detected as low as 0.04 ppm[93] while eye irritation began as low as 0.02 ppm.[83,93] Throat irritation may result with concentrations as low as 0.5 ppm;[83,93] however, these levels were not consistently irritating. At all concentrations the frequency of complaints decreased rapidly with time, indicating adaptation to the formaldehyde.[94] No lower airway symptoms were noted.[95] In 1983 Gamble published a thorough review of the literature, documenting the irritant effects of formaldehyde in various industries.[83] He reported that cohort studies of dress shop customers, garment factory workers, embalmers, and particle board workers have confirmed the irritant effects previously mentioned. Recently several cases of bronchial asthma have been reported to have resulted from exposure to formaldehyde

vapors.[96-99] Some authors regard this as a hypersensitivity reaction to the formaldehyde.[96,99]

There have been numerous complaints by consumers of products containing formaldehyde. Many of these were from residents of homes insulated with formaldehyde resins. Symptoms attributed to formaldehyde exposure in the home include breathlessness, headache, rhinitis, eye irritation, cough, colds, rash, malaise, sore throat, vomiting (in infants), drowsiness, and memory lapses.[47,83] Controlled studies[83] have shown a statistically significant increase in asthma, wheezing, or burning skin. Other symptoms which had higher (though not significantly) incidences were runny nose, rash, and dizziness.[83] At levels of formaldehyde above 0.3 ppm symptoms were linearly related to the concentration of formaldehyde. Below that level, reactions were highly variable. Gamble[83] summarized the irritant effects of formaldehyde by stating that

> ...there is no doubt formaldehyde is an upper respiratory tract and mucous membrane irritant. Exposures over 10 ppm are self-limiting because of the severe discomfort.... Levels of 4-5 ppm can be tolerated for 10-30 min. by some people....Most environmental and industrial exposures are probably around 3 ppm or less. It is also in this range that adaptation or acclimatization appears to occur....Eye, nose, and throat irritation can occur before odor detection. Irritation is slight at concentrations less than 1 ppm....The most convincing evidence for a toxic effect of formaldehyde on the lung are the cases of formalin asthma. Bronchial provocation in sensitized individuals produces delayed asthma responses that may last for weeks.

For years it was assumed that the only effects of exposure to formaldehyde were due to its irritational or sensitizing properties, and that the burning of the eyes, lacrimation, and general irritation of the respiratory passages would serve as a warning and prevent more severe effects from

occurring. However, this idea ignored research, begun as early as 1946 by Rapoport,[100] that indicated formaldehyde has both mutagenic and carcinogenic effects. Rapoport was the first to test for mutagenic effects of formaldehyde. He added it to the food of Drosophila melanogaster, the fruit fly, and obtained lethal mutations. In 1949 Auerbach[101] attempted to induce mutations in Drosophila by exposure to formaldehyde vapor. The attempts were unsuccessful, but when formaldehyde was added to the food, mutations occurred. She concluded that the action of formaldehyde was facilitated by one of the components of the food. Fraenkel-Conrat[68] reported in 1954 that formaldehyde inactivated Tobacco Mosaic Virus by reacting with the amino groups of the viral ribonucleic acid (RNA). Research on the effect of formaldehyde in the food on mutations in Drosophila over the next several years resulted in the following information: (1) Only larvae responded to treatment; no mutations were produced in adults. (2) Among larvae, only males responded. (3) In male larvae, sensitivity was restricted to early spermatocytes. (4) Formaldehyde produced all known types of mutations and chromosome rearrangements, with point mutations predominating.[37]

In the early 1960s Alderson[102-104] conducted a series of experiments in which he determined that formaldehyde had no mutagenic activity on Drosophila unless RNA was present in the treatment medium. He then discovered that the influence of RNA was due to the presence of adenylic acid. He stated that it did not appear to matter whether the adenylate was present as the free mononucleotide or bound in the ribonucleic acid polynucleotide. He suggested that adenylate acts as a mediating agent.

In reviewing the mechanism of the mutagenic activity, Alderson[105] found that formaldehyde reacts rapidly with free amino groups and more

slowly with hydrogen-bonded amino groups. Increasing the temperature not only increased the reactivity of the formaldehyde but also freed hydrogen-bonded amino groups of the purine and pyrimidine bases of the RNA. Formaldehyde reacted with these bases to form monomethylol derivatives ($-NH-CH_2OH$). Alderson stated that the main point of interest was the formation of dimeric forms of the bases through methylene bridges via the condensation of the monomethylol grouping on the amino group of one purine or pyrimadine with the free amino group of another. Since the sensitive period in the fruit fly is the time at which deoxyribonucleic acid (DNA) is being synthesized, he proposed that the mechanism for the mutagenic effect is based on the insertion of the wrong amino acid into the sequence of the DNA. Since RNA is single-stranded, the adenylate in RNA is available to bind with formaldehyde. DNA is normally wound in a double helix, and the adenylate is unvailable. As DNA is synthesized, it unwinds and the adenylate may be bound. This occurs, as in RNA, when a free amino group on a purine or pyrimadine base binds to formaldehyde to form the monomethylol derivative, which then binds to an adenylate molecule in the strand via a methylene bridge. This could cause a loop to form in the DNA strand, or form a cross link between adjacent strands, changing the order of the bases. This mistaken code order is subject to excision repair,[106] but unless the repair is completed before replication, the mistake is fixed into the gene code.[103]

Adenylate, along with adenosine-3'-phosphate, adenosine-5'-phosphate, and deoxyadenosine-5'-phosphate have been the only groups which promoted mutagenesis.[105] Experiments with other species have included grasshoppers,[107] fungi,[108] yeasts,[109] and bacteria.[106,110] All have been found to undergo mutations when exposed to formaldehyde. Various

substances, such as potassium cyanide,[111] dihydroxydimethylperoxide,[112] or

free radicals produced by reaction of formaldehyde with hydrogen

peroxide[111,113] may act to facilitate the process in each species.

The preponderance of evidence today favors the view that methylene

bridging forms crosslinks between two purines in adjacent DNA strands. This

is the primary genetic lesion produced by formaldehyde.[106,110] The damage

occurs at a moment when the DNA is unwound in single-strand form.[37]

In spite of the correlation between the mutagenic and carcinogenic

effects of chemicals, as late as 1977 there was no evidence for any

carcinogenic action by formaldehyde. Auerbach et al.[37] stated that "there

is no evidence for it and, indeed, hardly any attempt to test it (has been

made)." In fact, Alderson[114] emphasized that, in mice, formaldehyde

appeared to decrease the virulence and viability of tumor cells.

Then, in 1978, Muller et al.[115] reported that chronic topical

application of formalin produced leukoplakia and lesions which resembled

carcinoma-in-situ in rabbits. In 1980 Swenberg and associates[116] released an

interim report on a study conducted in conjunction with the Chemical

Industry Institute of Toxicology (CIIT), which showed that chronic exposure to

formaldehyde vapor produced tumors in the nasal turbinates of rats. In the

study, rats and mice were exposed to 0, 2, 6, or 15 ppm of formaldehyde

vapor for six hours per day, five days per week, for two years. There was

a sharp increase in the mortality rate among rats exposed to 15 ppm after 15

months. This increase "was due primarily to the occurrence of tumors in the

nasal turbinates." Many of the tumors were large osteolytic neoplasms, first

detected as a localized swelling over the nasal bones. The majority of these

were squamous cell carcinomas. No tumors were developed in rats at 0 or 2

ppm, three tumors at 6 ppm, and 95 tumors at 15 ppm. In all 240 rats and

240 mice were exposed to each dose level. Mice showed no tumors at less than 15 ppm, and only two at that level. Boreiko,[87] in a follow-up study, reported on the selectivity of the carcinogenic effects of formaldehyde for the nasal turbinates. Using $^{14}$C-formaldehyde, he showed that the inhaled formaldehyde was concentrated in the anterior portion of the nose, and was greatly diminished in the posterior nose and the naso-pharynx. The formaldehyde distribution was found to correlate strongly with the location of the tumors. Boreiko proposed that the high water solubility and chemical reactivity of formaldehyde combined with the obligatory nose-breathing of the rodents led to this deposition pattern. He theorized that the induction of carcinomas was a multistage process. The formaldehyde was shown to induce rapid cell turnover in the nasal turbinates, which normally have a low turnover rate.[117] The high rate was thought to be responsible for the fixation of oncogenic lesions in the DNA. This was supported by the fact that exposed rats first developed squamous metaplasia, followed by squamous hyperplasia, then carcinoma-in-situ, and finally invasive carcinoma. In contrast to this study, formaldehyde exposure of 10 ppm five times per week for life did not produce cancer in hamsters in another investigation.[118]

It is not possible to relate the results of animal studies directly to the reaction in humans. At present, knowledge of the effect of long-term formaldehyde exposure is limited to data from cohort studies on workers exposed to formaldehyde. In 1983 Halperin and associates[119] reported a case of squamous cell carcinoma in the nasal cavity of a 57-year-old man who had 25 years of exposure to low (0.2 to 1.2 ppm) concentrations of formaldehyde. His symptoms first appeared 21 years after his initial exposure. However, a direct relationship was not established since he had been exposed to other agents which are known to cause nasal cancer.

Other studies have reported no case of nasal cancer, and no increased rate of respiratory tract cancer in workers exposed to formaldehyde. Acheson et al.[120] reported the mortality experience of a cohort of 7680 men employed in six British chemical or plastics factories from 1965 through 1981. No deaths from nasal cancer or cancer of the nasopharynx were reported and no excess mortality was found for cancers of any type. There were no trends of increasing mortality with duration of work or interval since first exposure. The authors stated that evidence from occupational nasal cancer cases shows that the risk is evident only after a considerable interval since the first exposure. They stated that in the shoe and furniture industries no cases of nasal cancer have been reported where the interval between first exposure and diagnosis was less than 25 years. They concluded that it is premature to rule out a carcinogenic action of formaldehyde in nasal epithelium if a long minimum latent period is required.

Walrath and Fraumeni,[121] in a report on cancer rates among New York embalmers, found that they experienced significantly elevated mortality from skin cancer, with the excess primarily among those licensed for more than 35 years and those who began their employment at age 30 or later. They also found elevated mortality from kidney and brain cancers, though there was no excess mortality from cancers of the respiratory tract, including the nasal passages. They cautioned that these effects could be due, in part, to some component of embalming fluid other than the formaldehyde. Marsh[122] in 1983 published a mortality analysis on deaths among males employed in formaldehyde-related areas of a chemical plant. Overall he found "no statistically significant excesses or deficits in proportional mortality...among the formaldehyde-exposed group...(compared to others) from the same plant who did not have a month or more of

formaldehyde exposure." He found no trends or patterns in proportional mortality that could be directly linked to formaldehyde exposures. A case study of Danish doctors with lung cancer showed no relation between the occurrence of lung cancer and employment in pathology, anatomy, or forensic medicine.[123] Recently, however, Goldmacher and Thilley[124] showed that formaldehyde concentrations of 4.6 ppm can induce a significant number of mutations in human lymphoblast cell cultures.

The specifics of formaldehyde carcinogenicity in man have not been determined. Clary[118] postulated that formaldehyde may act in an epigenetic or cytotoxic manner, in which tissue damage occurs to such an extent as to cause cell death followed by cell regeneration. If this pattern recurs over a prolonged period, there is a continuous chance for a mistake to occur in the DNA replication cycle, resulting in a spontaneous increase in the rate of mutations, as suggested by Boreiko.[87] This mechanism implies a threshold level of exposure, below which there is only cell damage without overloading the normal repair mechanism. Clary pointed out that the data from Swenberg's study suggest a threshold for tumor formation. The risk factors and dose-response relationships have not been worked out. Much more information is needed before the mechanism can be detailed. Gibson[125] stated that "specific information on endogenous concentrations of formaldehyde in human tissues, cytological effects, occupational health effects, and mortality experience in humans must be gained to fully understand formaldehyde's potential for carcinogenesis in humans."

## GOVERNMENT REGULATIONS

As a result of increased awareness of the possible sources of formaldehyde exposure, the levels at which formaldehyde irritation occurs, and the possible carcinogenic effects of formaldehyde exposure, governmental bodies worldwide have established limits for exposure to formaldehyde. Following a survey[81] conducted from 1972 to 1974, NIOSH established a ceiling limit value of 1 ppm in the air of the workplace environment in any 30-minute sampling period.[88,126] The current standard is an eight-hour, time-weighted average concentration limit of 3 ppm, a ceiling of 5 ppm, and an acceptable maximum peak of 10 ppm for no more than a total of 30 minutes during an eight-hour shift.[127] The carcinogenic potential of formaldehyde was not well established when the regulations were issued, and was not considered in setting the acceptable levels; however, NIOSH recommended that formaldehyde be handled as a potential carcinogen, and that appropriate controls be used to reduce worker exposure.[127] It is conducting cohort studies to further refine the standards. Sundin[48] reported in 1982 that there was little doubt the CIIT findings had "colored the attitude of regulatory agencies and trade unions in Europe." Calls for a reduction in the permitted safety level have been widespread. Current and planned limits in Europe are shown on the following page:[48]

Exposure Limits for Formaldehyde in the Workroom
Environment in 1982

| Country | | Existing Value | Planned Value |
|---|---|---|---|
| Belgium | TLV | 2.0 ppm | --- |
| Denmark | TLV | 1.2 mg/cubic meter | 0.4 mg/cu.m |
| Finland | TLV | 2.0 ppm | 1.0 ppm |
| Germany | MAC | 1.0 ppm | --- |
| Holland | MAC | 2.0 ppm | --- |
| | TLV | 1.0 ppm(8 hr ave) | --- |
| Italy | TLV | 1.0 ppm | --- |
| Norway | TLV | 1.0 ppm | --- |
| Sweden | TLV | 1.0 ppm | 0.5 ppm |
| UK | TLV | 2.0 ppm | --- |

Eastern Europe

| | | | |
|---|---|---|---|
| DDR | TLV | 2.0 mg/cubic meter | --- |
| Hungary | TLV | 1.0 mg/cubic meter | --- |
| Poland | TLV | 2.0 mg/cubic meter | --- |
| Romania | TLV | 4.0 mg/cubic meter | --- |
| USSR | TLV | 0.5 mg/cubic meter | --- |
| Yugoslavia | TLV | 0.8 mg/cubic meter | --- |

TLV = Threshold Limit Value
MAC = Maximum Allowable Concentration
1.2 mg/cubic meter is approximately 1.0 ppm

It can be seen that the United States federal regulations permit exposure of higher levels than are permitted in Europe. Many U.S. cities and states have recently enacted more stringent requirements, especially dealing with the use of urea-formaldehyde foam insulation.[128]

# FORMALDEHYDE IN DENTISTRY

Formaldehyde is used in three basic ways in dentistry: (1) for sterilization and disinfection, (2) as formocresol in pulpotomies, and (3) as paraformaldehyde paste as a root canal filling material.

The use of paraformaldehyde powder or tablets for disinfection and sterilization of dental instruments and materials has been discussed. Formaldehyde gas is currently used in conjunction with a mixture of alcohols, ketones, and water in chemical vapor sterilization (Chemiclave). In this method a temperature of approximately $127^{O}C$ is maintained for 20 minutes at 20-25 psi. This has been shown to kill both spore-forming and non-spore-forming organisms.[129,130] The system has been approved by the Environmental Protection Agency[131] and by the Council on Dental Therapeutics of the American Dental Association.[132]

Formocresol was introduced in 1904 by Buckley[133] as a medication including equal parts of formalin and tricresol. The most popular commercial preparation of formocresol, Buckley's Formula, contains approximately 19% formaldehyde and 35% cresol in a glycerine and water vehicle. The formocresol pulpotomy technique used today was initially popularized by Sweet, who used it in the treatment of infected deciduous teeth.[134] The method consists of removing the coronal portion of the pulp down to the canal orifices, controlling hemorrhage, and applying a cotton pellet moistened with formocresol, for at least five minutes. The pellet is removed, and a creamy mix of zinc-oxide powder with equal parts formocresol and eugenol is placed into the pulp chamber against the amputated pulp. A cement base is placed over the paste and the tooth restored with amalgam or a crown.[135]

The details of the technique, its indications, and the ramifications of its use have been thoroughly discussed in the literature, and are reviewed in several textbooks.[25,133,135] Additionally, Berger[136] provided an excellent survey in 1972. The following are a few of the conclusions reached in the literature. When used in primary teeth with vital tissue, the technique has been shown to produce three distinct zones in the pulp:[137] (1) a layer of fixed tissue in contact with the formocresol, (2) a pale-staining zone in which the number of cells and fibers is greatly diminished, and (3) a zone containing a dense accumulation of inflammatory cells diffusing apically into normal tissue. There is no evidence of formation of a calcific barrier.[137] Doyle et al.[138] found that the pulp tissue in the apical one-third of treated healthy primary teeth remained vital, though calcific metamorphosis and complete replacement of the pulp tissue with granulation tissue have been demonstrated by other authors.[139,140]

The formocresol applied to the pulp in a pulpotomy is not confined to the root canal system. Accumulation of formocresol has been demonstrated in the pulp, dentin, periodontal ligament, and bone surrounding the apices of pulpotomized teeth.[141] It has been shown to be absorbed systemically and distributed throughout the body. Studies with $^{14}$C-labeled formocresol pulpotomies in dogs have discovered formaldehyde bound to tissue, predominantly in the kidneys, liver, lymph nodes, and lungs.[142,143] Pashley[142] found that the quantities of formaldehyde absorbed systemically were small and did not contraindicate its use.

Formocresol and paraformaldehyde have been shown to alter pulp tissue antigenically. When implanted in connective tissue or injected into the root canal, this altered autologous pulp tissue will induce a cell-mediated immune response in the host animal.[144-146] The immunologic responses vary in

different animals. Nishida et al.[147] elicited homologous antibodies to dental pulp treated with formalin in rabbits. Block et al.[148,149] detected humoral and cell-mediated responses to dog pulp tissue treated with high concentrations of formocresol or paraformaldehyde, while Van Mullem et al.[150] reported only a weak allergic response in guinea pigs. Eleazer et al.[151] found that a cell-mediated immune response is lacking in human dental pulp and the periapical tissue. Rolling and Thulin[152] reported that none of 128 children treated by formocresol pulpotomy developed skin sensitization to formocresol. Longwill et al.[153] noted significant lymphocyte transformation responses in 25 of 40 children who had received pulpotomies of various types. However, the responses were not specifically related to the use of formocresol. The authors stated that their results "should allay theoretical concerns of possible sensitization of children treated with a modest number of formocresol pulpotomies."

The use of paraformaldehyde pastes has also been extensively reviewed, and only a brief summary of the findings will be presented here. The first paraformaldehyde paste used in dentistry is believed to be that developed by Gysi in 1898.[136,154] His formula, Triopaste, contained about 17% paraformaldehyde, tricresol, creolin, and glycerin, and was developed for the purpose of mummifying the pulp. According to Rowe,[154] in the same year Abraham developed a paraformaldehyde-containing cement called Formagen for capping exposed pulps. More recently, Sargenti and Richter[155] developed a paraformaldehyde-containing paste, N2, for the purpose of filling the root canal space. The formula of N2 is not known precisely, but is believed to contain approximately 6.5% paraformaldehyde.[156] The studies conducted on paraformaldehyde pastes have involved implanting the materials, either directly or in tubes, into connective

tissue and bone of various animals, testing for cytotoxic effects of the materials on cell-cultures, and actually filling root canals with the pastes and observing the histologic effects.

The implant studies have tended to demonstrate that formaldehyde is extremely toxic to tissues it contacts. Guttuso[157] and Rappaport et al.[158] have found that N2 produced ulceration, severe inflammation, and necrosis when implanted in the subcutaneous tissues of rats. Langeland et al.[159] noted that the initial inflammation was severe and that it persisted, resulting in areas of dystrophic calcification. Friend and Brown[160] found, however, that though the initial inflammation was severe, it resolved rapidly. Unfavorable tissue responses were noted by numerous other investigators.[161-164]

Cell-culture studies have shown severe derangement of the cells exposed to paraformaldehyde pastes. Rappaport et al.[158] and Spangberg and Langeland[165] found N2 to be among the most toxic of the materials they tested in HeLa cell cultures. Other investigators have reached the same conclusion.[166,167] Paraformaldehyde paste has also been shown to inhibit cell respiration.[168]

Barker and Lockett[169] demonstrated that generally no periapical inflammation was produced when the canals were filled with N2 to the cemento-dentinal junction. When extruded into the periapical tissue, the N2 was encapsulated by fibrous tissue, but when in contact with bone, necrosis resulted. Brewer[170] found consistent ankylosis and bone necrosis in overfills of Sargenti paste, though no pathology resulted if the material was confined within the canal. Erausquin and Devoto[171] found that root canal treatment of rat molars using paraformaldehyde pastes resulted in severe and frequent ankylosis, and sequestration of alveolar bone. Snyder et al,[172] however,

found N2 less irritating than silver-containing cement when used in the root canals of dogs' teeth. The periapical areas of the dogs tolerated the N2 well, even when it was extruded beyond the apex. Langeland et al.[159] have shown that when used as an endodontic filling material after vital pulp extirpation, N2 induced inflammation and granuloma formation. Newton et al.[173] found that one year following treatment of teeth with pulpitis by the N2 method, periapical lesions had invariably formed, as a result of necrosis of tissues apical to the paraformaldehyde paste. s'Gravenmade[174] has stated that most actions of formaldehyde on pulpal tissue are reversible and that previously fixed tissues may break down, making long-term success unlikely.

METHODS AND MATERIALS

Experimentation involved three major areas: (1) the effectiveness of paraformaldehyde-powder sterilization of gutta-percha cones, (2) the adsorption of formaldehyde vapors onto the surface of gutta-percha cones, and (3) the measurement of residual formaldehyde gas produced during sterilization.

## PART 1 A

A pilot study was conducted to investigate the sterility and inherent antimicrobial properties of commercially packaged endodontic gutta-percha. Gutta-percha cones[a] of size 25 and size 80 were randomly selected and cultured immediately upon removal from previously unopened packages. Sterile cotton forceps were used to transfer a size 25 cone into each of nine 16 x 125 mm screw-top tubes[b] containing seven ml of Brain-Heart Infusion (BHI) broth,[c] and into each of nine screw-top tubes containing seven ml of thioglycollate medium.[d] Eighteen size 80 cones were cultured in the same manner. The BHI tubes were incubated aerobically and the thioglycollate tubes anaerobically at $37^{o}C$ for five days. The thioglycollate medium contained an oxygen-indicating agent, reazurin, which becomes pink in the presence of oxygen. Anaerobic conditions were maintained in a

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a. Hygienic Gutta-Percha Points, The Hygienic Corp., Akron, OH

b. Fisher Scientific Co., Pittsburgh, PA

c. BBL Microbiology Systems, Becton Dickinson and Company, Cockeysville, MD

d. Difco Laboratories, Detroit, MI

-45-

portable anaerobic system[a] (Figure 1). As an additional indicator of anaerobic conditions, a methylene blue dye strip was placed into the container. The strip remains white under anaerobic conditions, and becomes blue in the presence of oxygen.

The antimicrobial properties of the gutta-percha cones were tested against Staphylococcus aureus (IUPUI), Streptococcus salivarius (IUPUI), and Bacillus subtilis (ATCC 19659). Test organisms were obtained from the Department of Oral Microbiology, Indiana University School of Dentistry. Cells were prepared to obtain a final concentration of 100,000 cells/ml of suspension.[175] One-tenth ml of the standardized suspension (10,000 cells) was spread-plated over two BHI agar plates.[b] Three size 80 and three size 25 gutta-percha cones from unopened packages were placed equidistantly around the surface of each plate. The plates were incubated aerobically at $37^{o}$C for five days and examined for zones of inhibition of growth.

## Part 1 B

The effectiveness of paraformaldehyde-powder sterilization was tested in the following manner. Eighteen size 25 and 18 size 80 cones were selected at random from previously unopened packages and contaminated by immersion for 20 minutes in a suspension of Bacillus subtilis (globigii) spores.

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a. GasPak Disposable Anaerobic System (60460), BBL Microbiology Systems, Becton Dickinson and Company, Cockeysville, MD

b. BBL Microbiology Systems, Becton Dickinson and Company, Cockeysville, MD

A spore strip[a] was added to 500 ml of soil extract broth and incubated aerobically at 37°C for 35 days.[176,177] The spores were harvested by centrifugation at 6,000 rpm for 15 minutes at 4°C.[b] The supernatant was discarded and the spore pellet washed with 100 ml of normal saline. The procedure was repeated twice. The final cell concentration was adjusted with normal saline to an optical density of 0.3 at 520 nanometers.[c] This optical density was previously shown to correspond to a concentration of 100,000 cells/ml.[175] After immersion in the spore suspension, the cones were placed on sterile filter paper[d] inside a covered petri plate. The cones were allowed to dry for three hours at room temperature and aseptically transferred to a glass storage jar[e] which contained 12 compartments around a central well (Figure 2). The storage jar had been prepared in the following manner. Seven grams of paraformaldehyde powder,[f] weighed on an analytical balance,[g] were added to the jar's central well. The lid was placed on the jar and the formaldehyde gas was allowed to accumulate for forty-eight hours. The jar was then opened and the cones were transferred; three cones were placed into each compartment; six compartments contained size 25

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a. Spordex Bacterial Spore Strips, American Sterilizer Co., Erie, PA

b. Beckman Model J2-21, Beckman Instruments, Inc., Palo Alto, CA

c. Turner Model 350 Spectrophotometer, G.K. Turner and Associates, American Sterilizer Co., Erie, PA

d. Whatham #1 Filter Paper, W. & R. Balston LTD, England

e. Deep Liquid Sterilizer, Union Broach Co., Long Island City, NY

f. Sigma Chemical Co., St. Louis, MO

g. Mettler Instrument Corp., Hightstown, NJ

cones and six contained size 80 cones. To maximize the surface in contact with the formaldehyde vapors, the cones were not allowed to touch each other. The lid was then replaced. Cones were exposed for 30 minutes, 1 hour, 3 hours, 8 hours, 24 hours, and 48 hours. At each time interval, three cones of each size (all from the same compartment) were aseptically removed from the jar, and placed individually into culture tubes containing BHI broth. These tubes were immediately heat-shocked in an $80^{\circ}C$ bath for five minutes, and incubated aerobically for five days at $37^{\circ}C$.[176,177] Growth, as indicated by turbidity in the tubes, was then recorded. Positive cultures were gram stained and observed under a microscope. Aliquots (0.1 ml) from tubes in which there was no turbidity were transferred to culture tubes containing BHI broth. This was to check for possible residual effect of the paraformaldehyde. Thirty-six gutta-percha cones (18 of each size) were treated in an identical manner, except that no paraformaldehyde powder was placed in the jars. The viability of the spore suspension was confirmed by transferring 0.1 ml to three tubes containing BHI broth. These tubes were incubated aerobically for five days at $37^{\circ}C$. To determine how many spores were present on the surface of the gutta-percha cones, two cones of each size were immersed in the spore suspension for five minutes and dried on sterile filter paper. The cones were placed individually into test tubes[a] containing 10 ml sterile normal saline and vortexed for 10 seconds to remove the adherent spores. The separate suspensions from each of the four cones were serially diluted in a 10-fold fashion from $10^{-1}$ to $10^{-4}$. One-tenth ml

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a. Fisher Scientific Co., Pittsburgh, PA

of each dilution was spread over a BHI agar plate. The plates were incubated aerobically at 37$^o$C for five days, at which time the number of colony-forming units (cfu) was determined for each.

## Part 1 C

Because the surface characteristics of the gutta-percha cones may have influenced the effectiveness of the paraformaldehyde powder, ceramic "penicylinders"[a] were also tested (Figure 3). The penicylinders were ultrasonically cleaned in 0.5 N NaOH for 15 minutes, rinsed in continuously flowing tap water for 15 minutes, and placed on filter paper in petri dishes to dry. They were then autoclaved[b] at 270$^o$F under vacuum, and stored on sterile filter paper in covered sterile petri plates. A suspension of Bacillus stearothermophilus spores[c] was obtained in a manner similar to the B. subtilis suspension in Part 1 B, except that the B. stearothermophilus was grown in a soil extract broth and incubated at 56$^o$C for 35 days.[176,177] One-tenth ml of cells (10,000 cells at OD$_{520}$ of 0.3) was placed on each of two BHI agar plates, spread with a sterile glass rod, and incubated at 56$^o$C for five days to test the viability of the suspended spores. The remainder of the suspension was placed in a 250 ml flask, sealed with aluminum foil, and refrigerated until used.

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a. Fisher Scientific Co., Pittsburgh, PA

b. Vacamatic S, American Sterilizer Co., Erie, PA

c. Spordex Bacterial Spore Strips, American Sterilizer Co., Erie, PA

After confirmation of spore viability, the suspension was vortexed and poured into a sterile 500 ml beaker. The cylinders were aseptically placed into the suspension, immersed for one minute, transferred to dry sterile filter paper in sterile petri dishes and allowed to dry under vacuum for two days at room temperature. Twenty-four cylinders were placed into each of three compartmentalized glass storage jars, two cylinders per compartment (Figure 4), and the lids closed.

Each jar contained seven grams of paraformaldehyde powder in the central well. Twelve cylinders, one from each compartment, were aseptically sampled at periods of 1 hour, 2 hours, 4 hours, 6 hours, 12 hours and 24 hours. Six were placed immediately into individual tubes containing BHI broth, and six were transferred to a sterile petri dish. The formaldehyde was allowed to dissipate for one-half hour before these cylinders were placed into BHI tubes. All 12 tubes were heat-shocked in a water bath of $80^{o}C$ for five minutes, and placed in a $56^{o}C$ aerobic incubator. Growth, as indicated by turbidity, was judged after five days. At periods of 1 hour, 6 hours, and 24 hours, two cylinders which had not been exposed to the formaldehyde vapors were sampled along with the experimental groups to confirm continued spore viability.

**Part 1 D**

The ability of paraformaldehyde powder to prevent contamination of gutta-percha cones under clinical conditions was investigated. Using sterile cotton forceps, gutta-percha cones from previously unopened packages were

placed in a compartmentalized glass jar containing seven grams of paraformaldehyde powder. A total of 126 cones of each size (25 and 80) were selected at random, and divided into groups of 74 and 52. Each group occupied one section of the jar. The sections were labeled as to cone size and number present. The lid was placed on the jar which was then moved to a dental treatment room in active use. To simulate clinical use, the lid was removed from the jar for one-half hour each day. Using sterile cotton pliers, six cones were removed from each section at total exposure times of 12 hours, 1 day, 3 days, 7 days, 14 days, 30 days, and 60 days, and placed individually into sterile tubes containing BHI broth. Of each group of six, three were incubated aerobically, and three anaerobically[a] for five days at $37^{O}C$. Identical numbers of cones of each size were treated in the same manner as the experimental groups, without exposure to the paraformaldehyde powder. Positive cultures were indicated by turbidity in the tubes incubated up to five days.

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a. Anaerobic Chamber Model 1024, Forma Scientific, Marietta, OH

## PART 2

The second portion of the study was conducted in two phases. In the first, gutta-percha cones of size 25 and 80 were aseptically removed from previously unopened packages, weighed on an analytical balance, and placed into a compartmentalized jar containing seven grams of paraformaldehyde powder in the central well. One-half gram of size 25 cones was placed into each of six compartments, and an equal weight of size 80 cones was placed into each of the remaining six compartments (Figure 5). The lid was placed on the jar. After intervals of 1 day, 4 days, 7 days, 14 days, 30 days, and 60 days, the cones from one section of size 25 and one section of size 80 were removed, and placed in 2-dram jars,[a] one for each size. The cap was tightened firmly by hand, and sealed with parafilm.[b] The samples were taken to the Industrial Hygiene Laboratory of the Indiana State Board of Health, where the cones were placed in vials containing sodium bisulfite solution. The solution absorbed formaldehyde from the surface of the cones, and was analyzed using the chromotropic acid method.[178] A spectrophotometric assay was also conducted on the same liquid samples.[c] The transmission of light through the samples was compared with transmission through standards of known concentration, with the results given in ppm.

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a. Screw Cap Sample Vial, Fisher Scientific Co., Pittsburgh, PA

b. Parafilm M Laboratory Film, American Can Company, Dixie/Marathon, Greenwhich, CT

c. Hitachi 100-80 Spectrophotometer, Thomas Scientific, Philadelphia, PA

Identical amounts of the two sizes of gutta-percha were placed into compartments of an identical jar without paraformaldehyde powder, and sampled at the same times as the experimental groups.

In order to determine the rate at which adsorbed formaldehyde dissipates from the surface of cones, six one-half-gram samples of each size (25 and 80) cone were stored in a compartmentalized jar containing seven grams of paraformaldehyde. The lid was sealed in place for 60 days. At that time the cones were removed and placed into an identical jar which did not contain paraformaldehyde. Samples were taken at periods of 1 day, 4 days, 7 days, 14 days, 30 days, and 60 days following the transfer, and were assayed in the same manner as in the initial phase. An identical number of size 25 and size 80 cones was placed in an identical jar for 60 days without paraformaldehyde powder and sampled in the same manner as the experimental groups.

## PART 3

The amount of formaldehyde gas released when the lid is removed from the storage jar was determined by means of air sampling. The sampling device consisted of an impinger through which air is drawn by a battery-powered pump.[a] The pumps and impingers were provided by the Industrial Hygiene Laboratory of the Indiana State Board of Health. Each pump was calibrated at the laboratory to draw one liter of air per minute. The impinger contained a 1% solution of sodium bisulfite in which aldehydes in the air are dissolved as they flow through the liquid. The batteries were recharged after each use.[b] Background levels of formaldehyde were sampled in the outside air near the air intake on the roof of Indiana University School of Dentistry, and in the Graduate Endodontics Clinic of the school. These samples were taken at the close of the summer break, a time when the clinic had not been in use for one month. For each sampling, three pumps and three impingers were used. The outside air was sampled for a two-hour period, while the operatory air was sampled for one hour. In the operatory test the pumps with impingers attached were placed on the bracket tray in a position simulating a point six inches above the patient's face as he is receiving root canal treatment (Figure 6). Air sampled at this position

a.  MSA Portable Pump Model G, Mine Safety Appliances Co.,
    Pittsburgh, PA

b.  MSA Battery Charger Model 456059, MSA Co., Pittsburgh, PA

mimicked the environment to which the patient, operator, and assistant would be exposed during treatment. A second base-line sample was taken in the operatory after the clinic had been in use for two months.

To determine the amount of formaldehyde released when the lid was removed from the jar, three tests were conducted. In all three, the pumps and impingers were placed in the same position as in the background sampling. A glass storage jar containing seven grams of paraformaldehyde powder was placed on the instrument cart 24 inches from the pumps. This is the location of the jar during clinical use.

In the first test, the lid was removed from the jar for three 30-second periods over a time span of 30 minutes; simulating the amount of time required for an assistant to open the jar, remove gutta-percha cones as needed, and close the jar during an appointment in which the root canals are obturated. A second test, in which the lid was removed for the entire 30-minute period, simulated the clinical situation in which the lid is removed and not replaced until the conclusion of the fill appointment. In the final test, the lid was removed for five minutes and replaced. This was done 12 times during the course of a week in an effort to maximize the concentration of formaldehyde near the pumps. The total sample time was one hour. All indoor samples were made with the air conditioning system in use.

The impingers were removed from the pumps and sealed with parafilm. They were stored in a refrigerator until transported to the Indiana State Board of Health for analysis, using the chromotropic acid method and spectrophotometry. The analysis performed was identical to that done in PART 2.

RESULTS

## PART 1

### PART 1 A

After five days incubation, there was no growth in any of the tubes. The constant anaerobic environment was confirmed by both the reazurin and the dye strip. The gutta-percha cones did not inhibit the growth of any of the microorganisms. (Figure 7.)

### PART 1 B

Growth occurred in all but two control samples. These were both size 25 cones, taken at 30 minutes. Although killing occurred in some of the experimental tubes at every interval, at no period was sterilization accomplished in both size groups. The microscopic appearance of cells obtained from all the tubes in which growth occurred was consistent with that of B. subtilis. Subcultures from the tubes without growth exhibited no growth after incubation for five days. Incubation confirmed the viability of the spores in the suspension.

The viable cell count assay proved inconsistent and unpredictable because of sparse adherence to the surface of the cones.

The results are listed in Tables I-III.

PART 1 C

Sterilization of the penicylinders occurred only in samples taken after six hours of exposure to the formaldehyde vapor. Four of 12 cylinders (33.3%) sampled after 12 hours of exposure and one of 12 (8.3%) sampled after 24 hours yielded growth. While killing was demonstrated with exposures of less than six hours, 41.6% showed growth after four hours and 58.3% after two or fewer hours. Control cylinders demonstrated growth throughout the experiment. The results are shown in Table IV.

PART 1 D

There was no growth in any of the experimental or control samples at any time.

## PART 2

No adsorption of formaldehyde was detected on the 24-hour samples. Formaldehyde was first detected on size 25 cones after four days exposure, while none was noted on size 80 cones until exposure for seven days. After removal of the cones from the powder-containing jar, no formaldehyde was detected on any samples at any period. The analysis of formaldehyde adsorption is presented in Tables V and VI.

## PART 3

No formaldehyde was detected in the outside air. The level of detection was 1.0 ug. With a flow rate of 1.0 liter/min for two hours, 1.0 ug of formaldehyde would represent approximately 0.008 ppm. The background level of formaldehyde in the clinic was 0.04 ppm, whether the clinic was open or closed. No increase in formaldehyde was detected during any part of the experiment. The amount of formaldehyde detected is shown in Table VII.

FIGURES AND TABLES

FIGURE 1.   GasPak portable anaerobic system.  Tubes are placed
            in beaker within the sealed container to maintain an
            anaerobic environment.

FIGURE 2.   Deep liquid sterilizer used for storage of the
            gutta-percha cones.  The paraformaldehyde powder
            was placed in the central well and the cones in
            the surrounding 12 compartments.
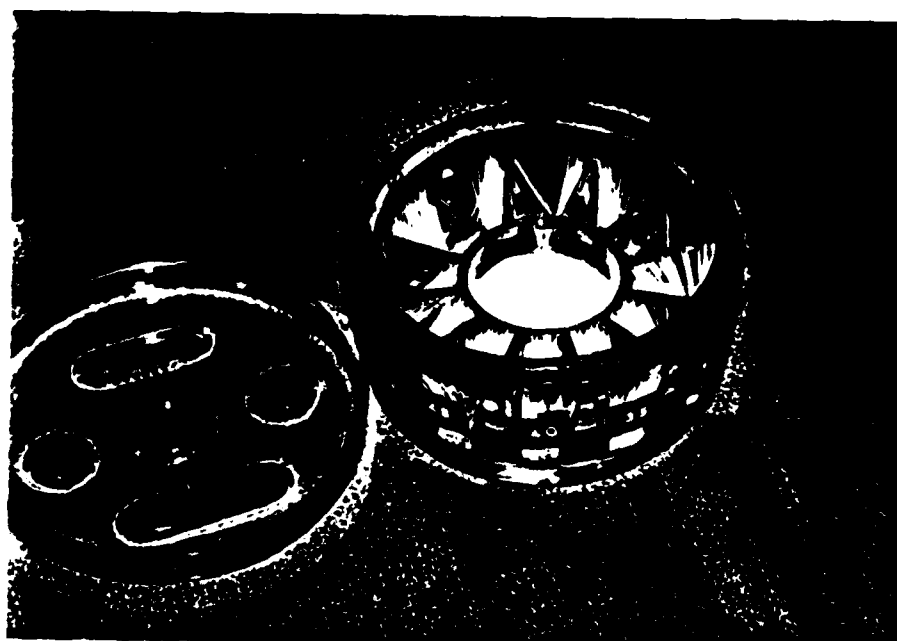
FIGURE 3.    Ceramic "penicylinders" upon which the B. stearothermophilus endospore suspension was dried.

FIGURE 4.    Ceramic "penicylinders" in glass storage jar with paraformaldehyde powder in the central well.
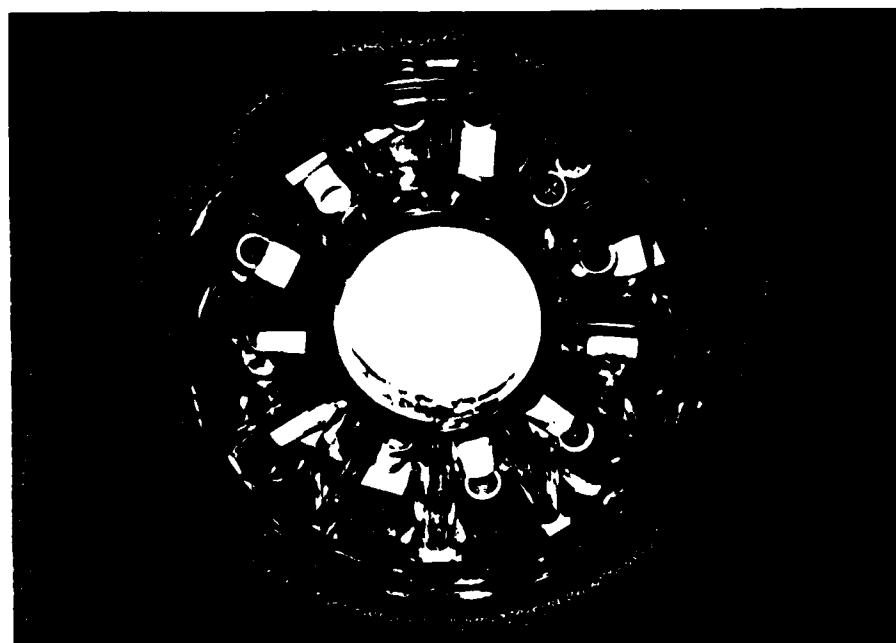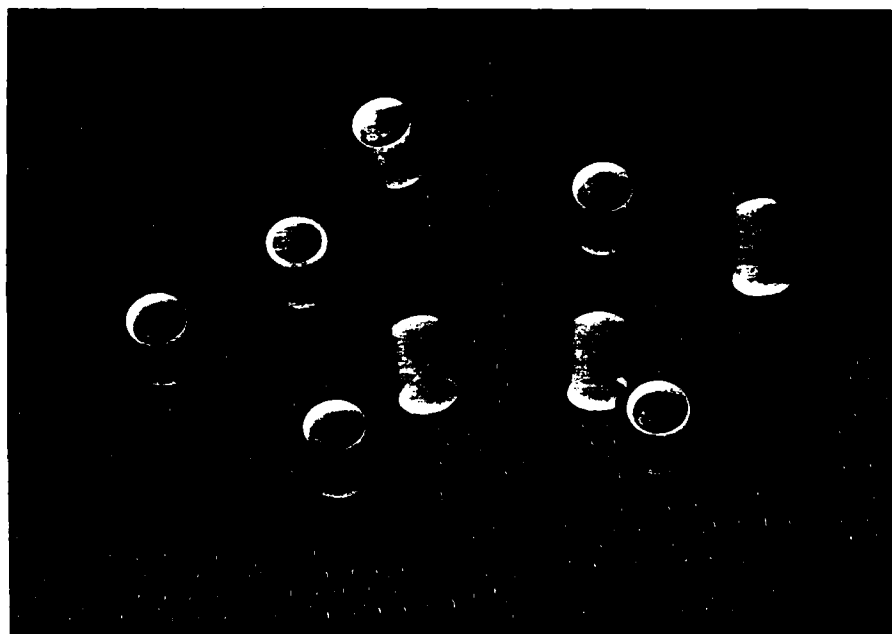
FIGURE 5.    Gutta-percha cones in glass storage jar with
paraformaldehyde powder in the central well.
One-half gram of cones was placed into each
compartment.

FIGURE 6.    Air sampling set-up in the endodontic clinic.
Three impingers with attached pumps were placed on
the bracket tray in a position to simulate the
clinical situation.
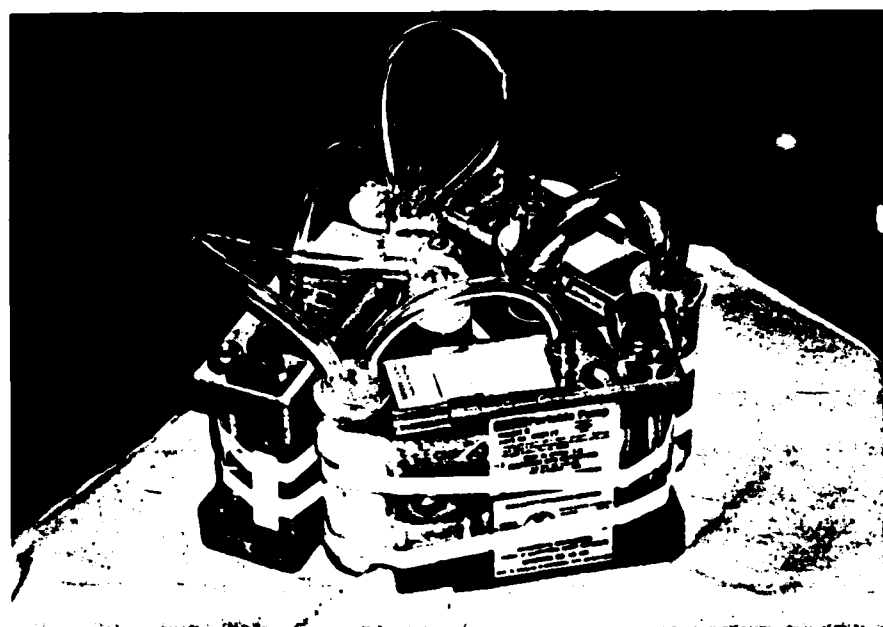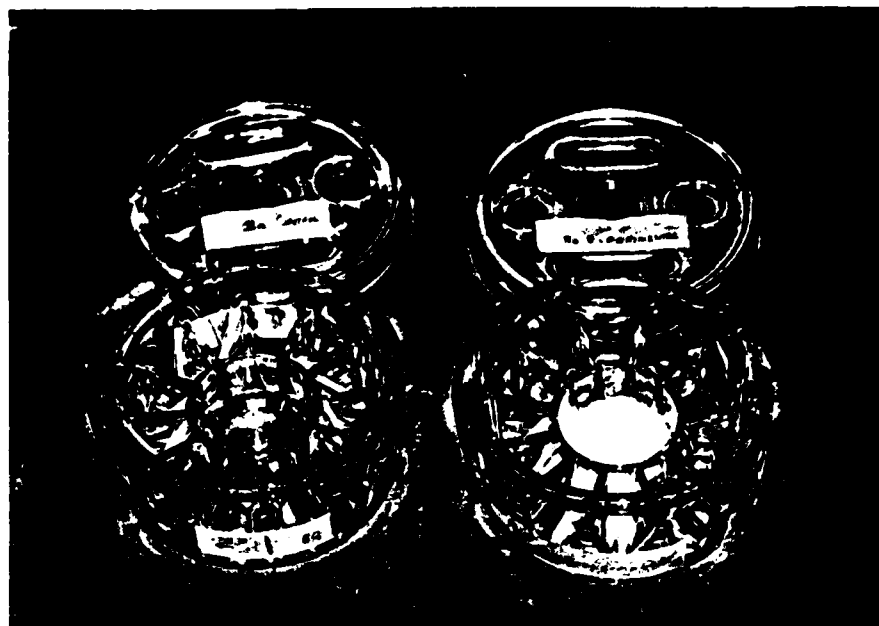
FIGURE 7.   Brain-heart infusion agar plate inoculated with
            B. subtilis.   The gutta-percha cones did not
            inhibit the growth of this or any of the organisms
            tested.
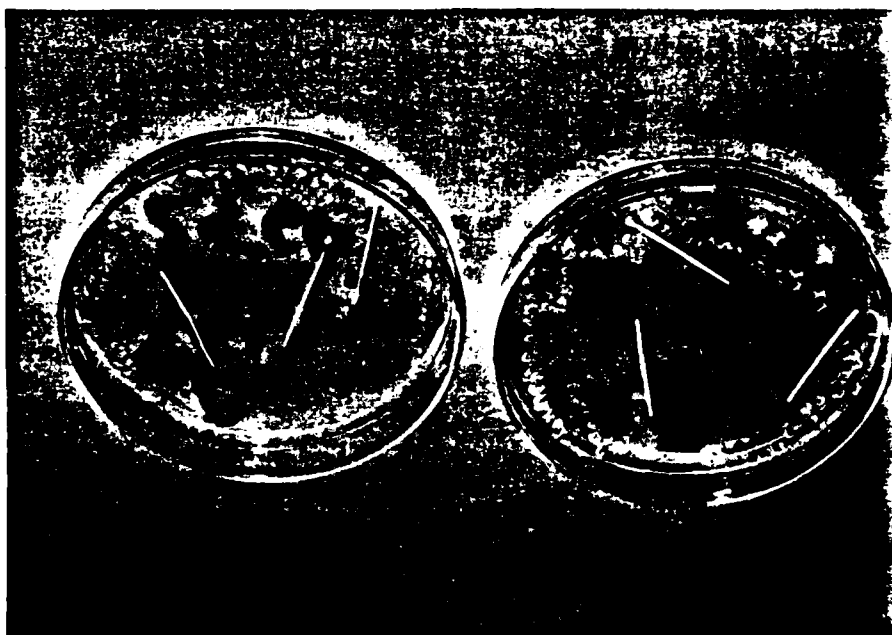
TABLE I

Paraformaldehyde powder sporicidal effectiveness

size 25 cones

| time | control* | experimental* |
|------|----------|---------------|
| 30 min | 1** | 3** |
| 1 hr | 3 | 2 |
| 3 hr | 3 | 1 |
| 8 hr | 3 | 0 |
| 24 hr | 3 | 3 |
| 48 hr | 3 | 0 |

\* three cones per group
\*\* number of positive cones after five days incubation

TABLE II

Paraformaldehyde powder sporicidal effectiveness

size 80 cones

| time | control* | experimental* |
|------|----------|---------------|
| 30 min | 3** | 1** |
| 1 hr | 3 | 1 |
| 3 hr | 3 | 2 |
| 8 hr | 3 | 1 |
| 24 hr | 3 | 1 |
| 48 hr | 3 | 1 |

\* three cones per group
\*\* number of positive cones after five days incubation

TABLE III

Spores adherent to immersed cones
(viable cell count)

| dilution | size 25 | size 80 |
|---|---|---|
| 10⁻⁴ | 1. no growth<br>2. no growth<br>3. no growth<br>4. no growth | 1. no growth<br>2. no growth<br>3. no growth<br>4. no growth |
| 10⁻³ | 1. no growth<br>2. no growth<br>3. 1 cfu*<br>4. 2 cfu | 1. no growth<br>2. no growth<br>3. 1 cfu<br>4. 1 cfu |
| 10⁻² | 1. no growth<br>2. no growth<br>3. 1 cfu<br>4. 1 cfu | 1. 2 cfu<br>2. 2 cfu<br>3. 2 cfu<br>4. 5 cfu |
| 10⁻¹ | 1. 1 cfu<br>2. 2 cfu<br>3. 3 cfu<br>4. 5 cfu | 1. 1 cfu<br>2. 12 cfu<br>3. 13 cfu<br>4. 17 cfu |

* colony-forming units

TABLE IV

Paraformaldehyde powder sporicidal effectiveness
penicylinders

| time | aerated | non-aerated | total |
|---|---|---|---|
| 1 hr | 1** | 6** | 7 |
| 2 hr | 3 | 4 | 7 |
| 4 hr | 2 | 3 | 5 |
| 6 hr | 0 | 0 | 0 |
| 12 hr | 2 | 2 | 4 |
| 24 hr | 0 | 1 | 1 |

\* total of 12 samples (six of each group)
\*\* samples showing growth after five days

TABLE V

Formaldehyde adsorption* by gutta-percha cones
size 25 cone

| time | per batch** | | per cone | |
|---|---|---|---|---|
| | experimental | control | experimental | control |
| 1 day | ---# | --- | --- | --- |
| 4 days | 3.6 | --- | 0.11 | --- |
| 7 days | 9.0 | --- | 0.27 | --- |
| 14 days | 3.3 | --- | 0.10 | --- |
| 30 days | 4.98 | --- | 0.15 | --- |
| 60 days | 10.5 | --- | 0.32 | --- |

\* amount in micrograms (ug)
\*\* 33 cones per batch
\# below the lower limit of detection (1.00 ug)

## TABLE VI

### Formaldehyde adsorption* by gutta-percha cones
### size 80 cones

| time | per batch** | | per cone | |
|------|--------------|---------|--------------|---------|
| | experimental | control | experimental | control |
| 1 day | ---# | --- | --- | --- |
| 4 days | ---- | --- | --- | --- |
| 7 days | 3.3 | --- | 0.41 | --- |
| 14 days | 3.3 | --- | 0.41 | --- |
| 30 days | 1.65 | --- | 0.21 | --- |
| 60 days | 3.0 | --- | 0.38 | --- |

\* amount in micrograms (ug)
** eight cones per batch
# below the lower limit of detection (1.0 ug)

TABLE VII

Air sampling for formaldehyde*

impinger

| area tested | A | B | C | net result** |
|---|---|---|---|---|
| outside air | ----# | ---- | ---- | ---- |
| Grad Endo Clinic ++ | ---- | 0.04 | 0.04 | 0.04 |
| Grad Endo Clinic && | 0.04 | 0.04 | ---- | 0.04 |
| Grad Endo Clinic @ 3 30-sec exp over 30 min | ---- | ---- | ---- | ---- |
| Grad Endo Clinic @ 30 min exp | ---- | ---- | ---- | ---- |
| Grad Endo Clinic @ 12 5-min exps. | 0.03 | 0.04 | ---- | 0.035 |

\* parts per million
** average of detected amounts
# below the limit of detection (1.00 ug)

++ background, clinics closed 4 weeks
&& background, clinics open 6 weeks
@ impinger 24" from paraformaldehyde

DISCUSSION

The gutta-percha cones were found to be sterile when they were removed from previously unopened packages. This is consistent with the findings of Kos et al.,[2] Doolittle et al.,[3] and Linke and Chohayeb.[4] As Friedman et al.[21] pointed out, the dental formulations of gutta-percha contain zinc oxide, gutta-percha, heavy metal sulfates, and small amounts of waxes or resins. Thus, the components of the gutta-percha cones appear to provide no nutrients to support bacterial growth and the only contaminants capable of surviving the manufacturing and packaging process probably are spore-formers. It was therefore not unexpected that the cones were uncontaminated. Most manufacturers make no special effort to sterilize the cones, but package them in a "clean" environment.[179,180] One brand, Indian Head, is available in vials which contain paraformaldehyde powder. These are advertised to be sterile, while none of the other brands make similar claims.

The cones did not demonstrate any inherent antibacterial properties against the test organisms. When the cones were placed on BHI agar plates inoculated with S. aureus, S. salivarius, and B. subtilis, no inhibition of growth occurred. This result conflicts with reports by Moorer and Genet[1,181] who attributed the inhibition of microbial growth by gutta-percha cones to the zinc-oxide component of the cones. They noted, however, that no inhibition of growth of S. aureus occurred when cones were placed on the surface of BHI agar. Only when "plate count agar" was used did inhibition occur. The authors used Kerr gutta-percha cones, which contain approximately 62% zinc oxide.[181] Bartels[182] found that cones made by Caulk, but not those made by S.S. White or Mynol, possessed a bacteriostatic action against gram (+) bacteria. Bartels attributed this action

to "some constituent of the gutta-percha which is variable in quantity." The manufacturers of Hygienic cones state that the concentration of zinc oxide in the cones is 67%.[179] Friedman and associates[21] found that the amount of zinc oxide in Mynol cones was approximately 59%. S.S. White and Caulk no longer manufacture gutta-percha and no information was found on the amount of zinc oxide that previous formulations contained. There does not seem to be a direct relationship between the amount of zinc oxide contained in the gutta-percha and the presence of bacteriostatic action noted by the various investigators. Further research into the source of the reported bacteriostatic properties of gutta-percha should be conducted, perhaps using several formulations of gutta-percha and various concentrations of zinc oxide without gutta-percha.

Since the cones tested in this study proved to be sterile when removed from the manufacturer's package, the clinician should direct his efforts toward the prevention of contamination during storage and use. The paraformaldehyde powder did prevent contamination by both aerobic and anaerobic bacteria for up to 60 days; however, cones stored without powder also remained sterile. It is not likely, therefore, that the paraformaldehyde was of significant value in preserving sterility. It appears that the paraformaldehyde powder may be unnecessary if the cones are sterile, stored in a covered container, and removed aseptically for use. In a dental school setting, an aseptic technique is not always followed while removing cones from storage. It is highly possible that some cones in the container may become contaminated while others are being removed. A sterilizing agent may be required in this situation. It is recommended that an investigation be conducted to determine whether contamination occurs while cones are removed from the storage jars and whether the use of paraformaldehyde

powder prevents contamination of cones in this manner. If the study shows that there is no contamination or that paraformaldehyde does not prevent contamination, its use should be discontinued.

Paraformaldehyde powder was inconsistent in its ability to sterilize gutta-percha cones contaminated with bacterial spores. This conforms to the findings of Nordgren,[47] the Committee on Formaldehyde Disinfection,[54] and Wigert et al.[58-60] The results contrast with those of Kantorowicz,[34] Buchbinder,[35] and El-Gammal and Mostafa,[55] all of whom used a method similar to that employed in this study but did not test against spore-formers.

Chi-square analysis of the results of the present study showed that there was no statistically significant difference in the number of samples with no growth when cones exposed to formaldehyde were compared to controls. Nor was there a significant difference between the action of size 25 cones and size 80 cones. There was a trend, however, toward a greater percentage of cones with no growth after eight or more hours of exposure as compared with exposures of three or less hours. Twelve of 18 (67%) of the 8-, 24-, and 48-hour experimental samples showed no growth, while only six of 12 (50%) exposed for three hours or less showed no growth (Tables I and II). Further investigations should include a much larger number of samples to increase the consistency of the results.

The spores did not adhere well to the gutta-percha surface and, as a result, inconsistent numbers of spores were deposited on the cones. Since it is possible that those cones which were coated with greater numbers of spores were not sterilized, while those with fewer spores were, gutta-percha did not provide a surface appropriate for testing the antimicrobial effects of formaldehyde gas. This surface property may be clinically helpful in resisting

contamination of cones by air-borne microbes. Other authors[3-5,28,30,32-35] have not noted any particular difficulty in inoculation of gutta-percha cones with bacterial suspensions. To improve adhesion of the spores, the cones could be treated by immersion in a solution of arginine, mucin, or artificial saliva to make them "sticky."[176]

The action of the formaldehyde vapors on the penicylinders was more consistent than that on the cones (Table IV). Fourteen of 24 cylinders (58%) sampled after exposure to the vapors for less than four hours demonstrated growth and five of 12 (42%) exposed for four hours tested positive. In six-hour samples no growth occurred in any of the cylinders, while four of 12 (33%) 12-hour specimens were positive and growth occurred in only one of the 12 24-hour samples (8%). These findings were subjected to chi-square analysis and showed a statistically significant effect of formaldehyde exposure (p<0.001).

The results indicate that a minimum exposure time is required for significant decontamination to occur. While sterilization can not be relied upon, exposure for at least six hours appears to greatly reduce the number of viable spores. As exposure time is increased, the killing rate increases. This is in agreement with the findings of Wigert et al.[60] that 15 hours of continuous exposure were required to kill B. subtilis spores. Phillips[45] recommended at least 12 hours to sterilize materials heavily contaminated with spores. Investigations should be conducted to determine more specifically the time required to kill spores deposited on gutta-percha cones. Samples should be taken more frequently between one and eight hours of exposure.

There appeared to be no difference in results between those cylinders placed immediately into the BHI tubes and those aerated for 30 minutes prior to culturing (Table IV). Approximately equal numbers of positive growth samples were found in each set, except at the one-hour sample. At that time all six placed immediately into the tubes were positive, while five of six aerated were negative. It cannot be determined from the results whether the cylinder samples in which no growth occurred were sterilized or were merely affected by release of residual formaldehyde. Further research should be undertaken to clarify this question. Control cylinders were positive throughout the experiment, confirming spore viability.

Formaldehyde was adsorbed onto the surface of the cones exposed to the paraformaldehyde vapors. This finding is consistent with the statement by Phillips[45] that a formaldehyde film is deposited on all surfaces exposed to the vapors released from the paraformaldehyde. However, Taylor et al.[52] found that no such film was formed unless the relative humidity was increased to 100%. The absence of a film does not rule out the presence of formaldehyde and, indeed, in the present study no film was noted. The relative humidity in the treatment room was not recorded. Since a normal comfort range was maintained throughout the experiment, the relative humidity was consistent with a clinical situation, well below 100%.

There appears to be a minimum period of exposure before adsorption of formaldehyde occurs on the surface of the cones. In this study no adsorption was noted in the 24-hour samples, and the first adsorption was noted after four days. No samples were taken between these periods. Testing at several intervals from one to four days should be conducted to determine when adsorption occurs. The accumulation of formaldehyde may be delayed by dissipation on opening the jar for sampling. This may also affect

killing times. In future experiments, separate jars should be used for each sample period, allowing uninterrupted build-up of formaldehyde prior to sampling.

Formaldehyde volatilizes and dissipates very rapidly, as demonstrated by the fact that no formaldehyde was detected on the cones within as little as 24 hours after removal of the powder. Further experiments are required to determine how quickly the formaldehyde leaves the cones. Whether this adsorption and rapid dissipation have any clinical effect is unknown. If the formaldehyde does not completely volatilize before insertion of the cone into the root canal, then formaldehyde may be released within the canal and, therefore, within the patient via the dentinal tubules or the apical foramen.

The permeability of the dentin to various drugs has been noted by numerous authors.[183-186] In an in-vitro study, Dankert et al.[183] examined the ability of formocresol and glutaraldehyde to diffuse through dentin and cementum. They stated that while glutaraldehyde showed no propensity to diffuse from the root canal, formocresol inhibited the growth of Staphylococcus epidermidis as far as 60 mm from the tooth. Repeated applications resulted in greater penetration; however, all diffusion was confined to the apical portion of the root. Marshall et al.[184] found that the apical dentin was the least permeable and all areas of the root canal showed a marked increase in permeability when irrigated alternately with 5.25% NaOCl and 3% hydrogen peroxide. If formaldehyde is released within a canal which has been prepared in this manner, it is possible that it may diffuse through the dentinal tubules and cementum to the surrounding tissues. Whether the formaldehyde diffuses or is bound within the sealer has not been determined and should be investigated.

There is also the possibility that an overextended fill might occur, in which case the formaldehyde-containing gutta-percha would come into direct contact with the periodontal ligament or alveolar bone. The effects of contact between formaldehyde and alveolar bone are well documented. As noted earlier, several authors[169-171] have reported that ankylosis and bone necrosis consistently result, with sequestration also occurring. N2 used as a filling material has induced inflammation and granuloma formation.[159] In a series of studies, Block et al.[148,149,187-189] have demonstrated that pulp tissue may be antigenically altered by reaction with formaldehyde. This tissue will produce a cell-mediated immune response when injected into the host animal. Thoden van Velzen and Feltkamp-Vroom[144] noted a similar response when autologous connective tissue was fixed in formaldehyde and implanted in rabbits. Whether the amount of formaldehyde released from gutta-percha cones, either through dentinal tubules or via an overextended fill, is sufficient to have a significant effect on the surrounding tissues and on the progress of healing following root canal treatment is unknown and should be investigated further.

There was no obvious adsorption pattern. Though the total formaldehyde concentration per batch of size 25 cones did tend to increase with time, that of size 80 cones actually decreased (Tables V, VI). The amount adsorbed per cone was consistently higher in size 80 cones than in size 25, indicating a direct relation between exposed surface area and amount of formaldehyde deposited.

The use of paraformaldehyde powder in the gutta-percha storage jars does not appear to increase staff exposure to formaldehyde if the room is well ventilated (Table VII). The outside air contained no detectable formaldehyde, while the background concentration of formaldehyde in the

operatory was 0.04 ppm. There was no change in the background level when the clinics were in use as compared to measurements taken during the summer break, indicating that the source of formaldehyde possibly was not a dental material or equipment. Though this level is well below the current NIOSH recommendation of 3 ppm, the source of this formaldehyde should be investigated. The sampling and analysis techniques utilized are sensitive to all aldehydes.[178] It is therefore possible that the background samples indicated the presence of an aldehyde other than formaldehyde. Further investigation should be undertaken to identify the compounds detected in the air samples. The levels of formaldehyde in the operatory, in both the background and experimental samples, were at the limits of detection for the equipment used. This may account for the variability among samples which were taken at the same time.

That formaldehyde was not detectable at a distance of 24" from the source is consistent with the findings of Kimmelman and Hillman,[126] who tested freshly opened containers of Sargenti N2 powder, formocresol, 10% formalin, and 37% formaldehyde. They noted that while readings taken one inch from the containers exceeded 15 ppm (the upper limit of detection), those taken at four inches were less than 0.2 ppm (the lower limit of detection). Measurements taken directly above 1.5 gm of Sargenti powder on a glass slab were also below the limits of detection. The authors concluded that formaldehyde vapors did not constitute a hazard, either to the office staff or to the patient. The results of the present study confirm this conclusion. In this study, ventilation was sufficient to remove the formaldehyde. It is possible that in poorly ventilated areas the concentration of formaldehyde may reach measurable levels. Given the multiple sources of

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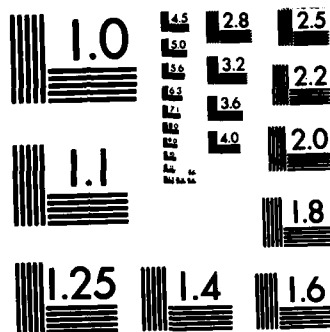
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daily formaldehyde exposure, and the possibility that formaldehyde is carcinogenic, even this small additional exposure may become significant.

Several methods of chairside decontamination of gutta-percha cones are available. Senia et al.[28] have shown that sodium hypochlorite (NaOCl) 5.25% effectively sterilizes gutta-percha after a 60-second immersion. Glutaraldehyde 2% has been shown to be effective against spores and hepatitis virus after 15 minutes exposure.[27] Both have been approved as disinfectants by the ADA Council on Dental Therapeutics.[29] Since there are currently no data as to the effects these agents have on gutta-percha, their use can not receive unqualified endorsement. Research should be conducted to determine whether the physical properties of the cones are affected by various liquid sterilizing agents. Additionally, the carry-over effects of the agents within the root canal system and in the periapical area should be investigated.

The use of paraformaldehyde powder for sterilizing gutta-percha cones is burdened with a series of shortcomings. It is not an effective sterilizing method. Cones contaminated with spores were not sterilized, even after as long as 48 hours, and other more reliable methods of chairside sterilization are available. While the powder did prevent contamination during simulated clinical exposure, storage of gutta-percha cones in a covered glass jar without the powder provided the same protection. The formaldehyde accumulates on the cones exposed to the formaldehyde vapors, with unknown clinical implications. Though it is probable that the amount of escaping formaldehyde gas is insufficient to be hazardous, it is prudent to eliminate this source of exposure to formaldehyde.

It is recommended that the use of paraformaldehyde powder for sterile storage of gutta-percha cones be discontinued, and an alternate method be

identified and investigated. Some methods which should be studied are the use of glutaraldehyde, sodium hypochlorite in various concentrations, and ultra-violet light. Paraformaldehyde powder should remain in use, at least in the dental school setting, until the suggested research is completed.

SUMMARY AND CONCLUSIONS

Since the chain of asepsis in endodontic treatment can be broken by obturation with contaminated gutta-percha cones, this investigation was undertaken to determine whether the use of paraformaldehyde powder for the sterile storage of gutta-percha is necessary, safe, and effective.

Size 25 and 80 gutta-percha cones were aseptically removed from previously unopened containers and cultured aerobically and anaerobically. Brain-Heart Infusion (BHI) broth was the medium used for aerobic incubation, while thioglycollate broth was used for the anaerobic samples. After incubation for five days at $37^{o}C$, no growth occurred in any of the 36 randomly selected samples.

The antimicrobial properties of the gutta-percha cones were tested against Staphylococcus aureus (IUPUI), Streptococcus salivarius (IUPUI), and Bacillus subtilis (ATCC 19659). Test organisms were prepared to obtain suspensions containing 100,000 cells/ml. One-tenth ml of the suspension was spread-plated over two BHI agar plates. Three size 25 and three size 80 gutta-percha cones from unopened packages were placed equidistantly around the surface of each plate. The plates were incubated aerobically for five days at $37^{o}C$. The cones did not inhibit the growth of any of the test organisms.

The effectiveness of paraformaldehyde powder sterilization was tested against gutta-percha cones and penicylinders contaminated by immersion in bacterial endospore suspensions. The cones, 18 size 25 and 18 size 80, were randomly selected from unopened packages and contaminated by immersion for 20 minutes in a suspension of Bacillus subtilis (globigii) spores. The concentration of the suspension had been determined to be 100,000 cells/ml. The 84 sterile penicylinders were immersed for one minute in a suspension of

Bacillus stearothermophilus at the same cell concentration. Both the cones and the cylinders were allowed to dry at room temperature under a vacuum and were then aseptically transferred to sterile glass storage jars. The jars contained 12 compartments around a central well. The jars of the experimental groups contained seven grams of paraformaldehyde powder in the central well. Control groups of cones and cylinders were treated in a manner similar to the experimental groups, without exposure to paraformaldehyde powder. Both the experimental and control groups were sampled after various intervals and placed in tubes containing BHI broth. The cones were incubated aerobically at $37^{\circ}C$ and the penicylinders at $56^{\circ}C$ for five days. The gutta-percha cones did not provide an adequate surface for spore adherence, and the paraformaldehyde showed inconsistent results. While the paraformaldehyde showed a statistically significant amount of killing on the penicylinders, sterilization was not consistently achieved.

The ability of paraformaldehyde powder to prevent contamination of gutta-percha cones under clinical conditions was investigated. A total of 126 cones of each size (25 and 80) were selected at random, divided into groups of 74 and 52, and aseptically transferred to a compartmentalized storage jar. The central well contained seven grams of paraformaldehyde powder. An identical number of cones was placed in a similar jar without the paraformaldehyde. The lid was removed from each jar for 30 minutes each weekday to simulate clinical use. At total exposure times of 1 day, 4 days, 7 days, 14 days, 30 days and 60 days six cones were removed and placed into tubes containing BHI broth. Three samples were incubated aerobically and three anaerobically for five days at $37^{\circ}C$. No growth occurred in any of the tubes at any time.

The adsorption of formaldehyde onto the surface of gutta-percha cones was studied by exposing size 25 and 80 cones to the formaldehyde generated in a glass storage jar by seven grams of paraformaldehyde powder. One-half gram samples of each size cone were removed after total exposure times of 1 day, 4 days, 7 days, 14 days, 30 days and 60 days. The samples were analyzed, using a spectrophotometer, for the amount of formaldehyde released. Identical batches of cones were exposed to paraformaldehyde powder for 60 days in another jar. The powder was then removed and the cones were analyzed at the above intervals to determine the rate of formaldehyde dissipation. The formaldehyde adsorption onto the cones occurred after a minimum exposure of more than 24 hours and less than four days. The adsorption appeared to be related to the surface area exposed to the vapors. Twenty-four hours after removal of the powder, no formaldehyde was detected on the surface of the cones.

The effect of the use of paraformaldehyde powder on the air of the dental operatory was studied. Air samples were taken of the outside air and in the Graduate Endodontics Clinic. No formaldehyde was detected in the outside air. Background levels of formaldehyde in the clinic registered 0.04 ppm. This level was constant whether or not the clinic had been in use. Samples taken in the clinic while the formaldehyde jar was open showed no detectable levels of formaldehyde at a distance of 24" from the source, unless the exposure was broken into a series of extremely short (five minutes) periods. Even at that, the exposure reached only 0.035 ppm, which is equal to the normal background level.

It was concluded that the gutta-percha cones were sterile as removed from the package and that they did not possess any inherent antimicrobial activity. Therefore, the primary concern should be the prevention of

contamination of the gutta-percha during storage and use. Paraformaldehyde powder was found to be an ineffective sterilizing agent, though it did exhibit sporicidal activity. Cones stored in a covered glass jar without the paraformaldehyde did not become contaminated during 60 days of simulated clinical use. Formaldehyde was adsorbed to the surface of cones after exposure to the vapor for four days. Twenty-four hours after removal of the cones from the powder, no formaldehyde was detected. Since formaldehyde is a suspected carcinogen, even the small amount of formaldehyde which was detected on the cones and in the air should be avoided if possible. The effects of formaldehyde-containing gutta-percha cones on healing after root canal therapy should be examined. In addition, an investigation should be conducted to determine whether any sterilizing agent is necessary for the storage of gutta-percha. If such an agent is required, an alternative to paraformaldehyde should be identified and evaluated.

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CURRICULUM VITAE

## James Raymond Higgins

| | |
|---|---|
| June 6, 1948 | Born to James and Delores Higgins, Los Angeles, California |
| May 27, 1970 | Elected to membership in Phi Beta Kappa, Miami University, Oxford, Ohio |
| June 14, 1970 | B.A., cum laude, Miami University, Oxford, Ohio |
| June 1970 to July 1975 | Service in the United States Navy |
| August 1975 to May 1979 | D.D.S., with Distinction, Indiana University School of Dentistry, Indianapolis, Indiana |
| May 19, 1979 | Married Elizabeth Aileen Harlow |
| June 1979 to July 1980 | Dental General Practice Residency USAF Hospital, Davis-Monthan AFB, Arizona |
| July 1980 to July 1983 | General Dental Officer, George AFB, California |
| August 1983 to July 1985 | M.S.D. Program, Endodontics, Indiana University School of Dentistry, Indianapolis, Indiana |

## Professional Organizations

American Dental Association
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Harry J. Healy Endodontic Study Club
Omicron Kappa Upsilon Honorary Society

ABSTRACT

# A MICROBIOLOGIC AND SPECTROPHOTOMETRIC INVESTIGATION

## OF THE USE OF PARAFORMALDEHYDE POWDER IN THE

## STERILIZATION OF GUTTA-PERCHA CONES

by

James R. Higgins

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Indianapolis, Indiana

This investigation was undertaken to determine whether the use of paraformaldehyde powder for the storage of gutta-percha is necessary, safe, and effective.

Gutta-percha cones were aseptically removed from unopened containers and incubated aerobically and anaerobically for five days at $37^{o}C$ in appropriate media. No growth occurred in any of the 36 randomly selected samples.

The antimicrobial properties of gutta-percha cones were tested against Staphylococcus aureus, Streptococcus salivarius, and Bacillus subtilis. Test organisms were spread-plated onto Brain-Heart Infusion (BHI) agar plates, and gutta-percha cones placed onto the surface of the plates. After incubation for five days at $37^{o}C$ the cones did not inhibit the growth of any of the organisms.

The effectiveness of paraformaldehyde powder sterilization was tested against gutta-percha cones and penicylinders contaminated with bacterial endospore suspensions. The contaminated cones and cylinders were placed in glass storage jars which contained paraformaldehyde powder. After various

exposure intervals, samples were placed into tubes containing BHI broth for aerobic incubation; the cones at $37^\circ C$ and the cylinders at $56^\circ C$ for five days. The gutta-percha cones provided a poor surface for spore adhesion, and no statistically significant sporicidal action was demonstrated. While significant ($p<0.001$) killing was demonstrated on the penicylinders, sterilization was not consistently achieved.

Cones were placed into glass storage jars containing paraformaldehyde powder in the central well, and sampled at various times over a 60-day period. Formaldehyde adsorption onto exposed gutta-percha was demonstrated, using spectrophotometry, after only four days exposure. Another set of cones was exposed to the formaldehyde vapors for 60 days and then tested. Spectrophotometric analysis revealed that all the formaldehyde had dissipated within 24 hours after removal of the cones from the powder.

Air sampling revealed that the level of formaldehyde in the clinic did not increase as a result of the use of paraformaldehyde powder sterilization.

The use of paraformaldehyde powder for sterilization was found to be ineffective against bacterial endospores. Further research is suggested to determine whether any sterilization agent is required for the storage of gutta-percha. Although the levels of formaldehyde exposure did not increase as a result of the use of paraformaldehyde powder, it was considered prudent to eliminate this source of further exposure due to the possible carcinogenic effects of formaldehyde. It is recommended that an alternative to paraformaldehyde be identified and evaluated.

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